Shell Chemicals

November 14, 2006

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To Whom It May Concern:

Shell Chemical LP does not currently manufacture or import any of the substances listed at 71 FR 47130-47141 (16 August 2006). However, a search of our files produced a number of unpublished reports that the Agency may find useful. These are as follows:

1) Contact Hypersensitivity to o-cresylglycidylether in Albino Guinea Pigs, Maximization-Test. 25 February 1991.
2) Monobutyl-p-cresol: Bacterial mutagenicity studies. December 1992,
3) Butyl-p-cresol: physicochemical properties. June 1995.
4) Toxicity of WL 43775 Intermediates: Acute Toxicity, Skin and Eye Irritancy and Skin Sensitization Potential of m-bromobenzaldehyde. March 1977.
5) Toxicity Studies with Mining Chemicals: *in vitro* Genotoxicity Studies with sodium isopropyl xanthate. 4 July 1981.
6) Toxicology of Mining Chemicals: Acute Toxicity, Skin and Eye Irritancy and Skin Sensitization Potential of sodium isopropyl xanthate. March 1978.
7) Cyclopentadiene: I. Six-hour LC50 Vapor Inhalation Study on Mice; II. Nine-day Vapor Inhalation Study on Mice. 16 November 1981.
8) Biodegradation of m-phenoxybenzoic acid, pentaerythritol and methanesulfonyl chloride in the Presence of a Soft Co-substrate. 2 September 1977.

Redacted copies of the studies are attached. If you have any questions, please contact me at (713) 241-0032.

Very truly yours,

Michael Hulse
HSSE/SD
Shell Chemical LP

R C C

RESEARCH & CONSULTING COMPANY AG

RCC PROJECT 272913

CONTACT HYPERSENSITIVITY TO

O-CRESYLGLYCIDYLETHER (CGE)

IN ALBINO GUINEA PIGS

MAXIMIZATION-TEST

REPORT

Data Requirements	:	OECD Guidelines 406 (May, 1981) EEC Guidelines B.6 (March, 1984)
Authors	:	[redacted]
Performing Laboratory	:	RCC, Research & Consulting Company AG, P.O. Box, CH-4452 Itingen

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APPENDIX

PREFACE

GENERAL

Title	Contact Hypersensitivity in Albino Guinea Pigs. Maximization-Test.
Sponsor	APME Represented by [redacted] c/o Ciba-Geigy AG Postfach CH-4002 Basel
Addressee	[redacted] Ciba-Geigy AG PS 1.2, Stab Toxikologie AKP R-1030.1.05 CH-4002 Basel
Monitoring Scientist	[redacted] Ciba-Geigy AG
Testing Facility	Research & Consulting Company AG CH-4452 Itingen
RCC Project Number	272913
Test Article	O-CRESYLGLYCIDYLETHER (CGE)
Test System	Guinea pigs

PROJECT STAFF

Study Director	[redacted]
Technical Coordinators	[redacted]
Study Veterinarian	[redacted]

SCHEDULE

Pretest start	August 14, 1990
Acclimatization	September 24 to 30, 1990
Treatment start	October 1, 1990
First challenge	October 22, 1990
Second challenge	November 5, 1990
Termination	November 8, 1990
Reported	January 25, 1991 / kla

ARCHIVING

Research & Consulting Company AG, CH-4452 Itingen will archive the following data for at least 10 years:
raw data, protocol and report, duplicate of report and test article reference sample.

PROJECT STAFF SIGNATURES

Study Director : [redacted]

date: Jan. 25, 1991

Technical Coordinator : [redacted]

date: Jan. 25, 1991

Managing Director : [redacted]

date: Jan. 25, 1991

QUALITY ASSURANCE UNIT

R C C, REGISTRATION & CONSULTING COMPANY AG, CH-4452 ITINGEN

S T A T E M E N T

RCC
PROJECT NUMBER : 272913

TEST ARTICLE : O-CRESYLGLYCIDYLETHER (CGE)

STUDY DIRECTOR : [redacted]

TITLE : Contact Hypersensitivity in Albino Guinea Pigs. Maximization-Test.

Study procedures were periodically inspected and this report was audited by the Quality Assurance Unit. The dates are given below.

Dates of QAU Inspections / Audits	Dates of Reports to the Study Director and to Management
09.08. 1990 05.10. 1990 01.02. 1991	09.08. 1990 05.10. 1990 01.02. 1991

Manager, Quality Assurance Unit [redacted]

Date: Feb. 21. 1991

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

RCC PROJECT NUMBER	:	272913
TEST ARTICLE	:	O-CRESYLGLYCIDYLETHER (CGE)
STUDY DIRECTOR	:	L. Ullmann
TITLE	:	Contact Hypersensitivity in Albino Guinea Pigs. Maximization-Test.

Stability of the test article dilution is excluded from this Statement.

To the best of my knowledge and belief, the study described in this report was conducted in compliance with the following Good Laboratory Practice Standard:

Environmental Protection Agency; Good Laboratory Practice Standards; Final Rule, U.S.A. Federal Register, Vol. 54, No. 158, August 17, 1989.

Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986.

OECD Principles of Good Laboratory Practice, Paris, France, adopted May 12, 1981.

Study Director [redacted]

Date: Jan. 25, 1991

Sponsor APME

.....

Date:

TEST GUIDELINES

There were no circumstances that may have affected the quality or integrity of the data.

The study procedures described in this report are based on the following guidelines:

Directive 84/449, EEC B.6. "Acute Toxicity - Skin Sensitization", March 1984.

OECD Guidelines for Testing of Chemicals, Section 4, number 406, "Skin Sensitization", adopted May 12, 1981.

Magnusson B. Kligman A.M., 1969.
The identification of contact allergens by animal assay. The guinea pig maximization test. J. Invest. Dermatol. 52: 268-276.

SUMMARY OF PROTOCOL AMENDMENTS

- First Amendment:

  Schedule dates were completed in the protocol.

- Second Amendment:

  Number of animals used in the test was changed and distribution of animals for the intracutaneous and epicutaneous applications was added to the protocol;

  Stability of test article in oleum arachidis and vaseline was excluded from Statement of Compliance;

  The test article preparation was changed;

  Because three epidermal pretest studies were performed the epidermal pretest paragraph was adopted;

  The intradermal induction procedure was changed;

  On request of the sponsor the vehicle (vaseline) used for the first challenge was changed for the second challenge and replaced by oleum arachides.

SUMMARY

To assess the allergenic potential of O-CRESYLGLYCIDYLETHER (CGE) in albino guinea pigs the Maximization-Test of B. Magnusson and A.M. Kligman (1969) was used. Ten animals (5 males, 5 females) were used as control group and 20 animals (10 males, 10 females) were used as test group.

The study was conducted between August 14th and November 8th, 1990 at the RCC laboratories in CH-4452 Itingen.

RESULTS

The highest non-irritating test article concentration used for the both challenge applications was 1%.

POSITIVE ERYTHEMA REACTIONS AFTER FIRST CHALLENGE PROCEDURE

	after 24 hours positive / total % positive of total	after 48 hours positive / total % positive of total
CONTROL GROUP		
O-CRESYLGLYCIDYLETHER (CGE) (left flank)	0 / 10 0	0 / 10 0
* vehicle only (right flank)	0 / 10 0	0 / 10 0
TEST GROUP		
O-CRESYLGLYCIDYLETHER (CGE) (left flank)	16 / 20 80	14 / 20 70
* vehicle only (right flank)	0 / 20 0	0 / 20 0

* Vaseline was used as vehicle.

SUMMARY cont'd

POSITIVE ERYTHEMA REACTIONS AFTER THE SECOND CHALLENGE PROCEDURE

	after 24 hours positive / total % positive of total	after 48 hours positive / total % positive of total
CONTROL GROUP		
* vehicle only (left flank)	0 / 10 0	0 / 10 0
TEST GROUP		
O-CRESYLGLYCIDYLETHER (CGE) (right flank)	1 / 20 5	0 / 20 0
* vehicle only (left flank)	0 / 20 0	0 / 20 0

* Oleum arachides was used as vehicle.

No toxic symptoms were evident in the guinea pigs of neither the control nor test group.

No death occurred.

CONCLUSION

For the interpretation of the allergenic potential of the test article the results received after the first challenge were used.

From the results described above "strong" allergenic potency of the test article O-CRESYLGLYCIDYLETHER (CGE) was concluded. The results were interpreted according to the rating of Magnusson and Kligman (1969).

According to EEC (European Economic Community) classification criteria described in guidelines 83/467, September 16, 1983 and 67/548, May 1987, this test article is considered to be a sensitizer.

OBJECTIVE

PURPOSE AND RATIONALE

The purpose of this skin sensitization study was to assess the allergenic potential of O-CRESYLGLYCIDYLETHER (CGE) when administered to the skin of albino guinea pigs.

This study should provide a rational basis for risk assessment of the sensitizing potential of the test article in man.

MATERIALS AND METHODS

Experimental Design

TEST SYSTEM

Test system	Ibm: GOHI; SPF-quality guinea pigs (synonym: Himalayan spotted)
Rationale	Recognized by the international guidelines as the recommended test system, (e.g. OECD, EEC).
Source	BRL, Biological Research Laboratories Ltd. Wölferstrasse 4 CH-4414 Füllinsdorf
Total Number of animals	22 males (7 males used for pretest) 22 females (7 females used for pretest)
Age at Acclimatization Start	males : 7 weeks females: 8 weeks
Body Weight at Acclimatization Start	males: 308 - 350 g females: 324 - 345 g
Identification	By unique cage number and corresponding ear tags.
Randomization	Randomly selected at time of delivery.
Acclimatization	One week under test conditions after veterinary examination.

The animals were distributed as follows:

5 males, 5 females for the control group and 10 males, 10 females for the test group. One male, one female for the intracutaneous (I.C.) pretest and 6 males, 6 females for the epicutaneous (E.C.) applications.

GROUPS	ANIMAL NUMBERS MALES	FEMALES
1 Control Group*	61 - 65	76 - 80
2 Test Group*	66 - 75	81 - 90
3 I.C. Pretest*	691	796
4 E.C. Pretest*	692 - 693 493 - 494 1; 3	797 - 798 497 - 498 2; 4

* Oleum arachides was used as vehicle for the intracutaneous and second challenge applications and vaseline for the other epicutaneous applications.

A control group (Formaldehyde-solution) is tested twice a year for sensitivity check of the guinea pig strain (see Appendix E). The most recent test was run from April 23 to May 24, 1990 (RCC 270066).

HUSBANDRY

Room No.: 135

Conditions

Standard Laboratory Conditions
Air-conditioned with 10-15 air changes per hour and hourly monitored environment with a temperature of 22 ± 3 degrees centigrade, a relative humidity between 40-70 %, 12 hours artificial fluorescent light/12 hours dark, music during the light period.

Accommodation
Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz).

Diet
Pelleted standard Kliba 342, Batches 59/90 and 60/90 guinea pig breeding/maintenance diet ("Kliba", Klingentalmühle AG, CH-4303 Kaiseraugst), ad libitum. Results of analyses for contaminants are included in this report.

Water
Community tap water from Itingen, ad libitum. Once weekly additional supply of ascorbic acid via the drinking water. Results of analysis for contaminants are included in this report.

TEST ARTICLE

Identification	O-CRESYLGLYCIDYLETHER (CGE)
Description	liquid
Batch Number	DC 1294.1
Purity	98.9%
Stability of test article	stable in closed containers until June 1991
Stability of test article dilution	unknown; excluded from Statement of Compliance
Storage Conditions	at room temperature, in the dark
Safety precautions	Gloves, goggles and face mask were sufficient to assure personnel health and safety.

TEST ARTICLE PREPARATION

The test article and vehicle were placed into a glass beaker on a tared Mettler PK 300 balance. For intracutaneous and second challenge application weight/weight dilutions were prepared using a homogenizer. Homogeneity of the test article in vehicle (oleum arachidis) was maintained during treatment using a magnetic stirrer. For the other epicutaneous applications weight/weight dilutions were prepared with vaseline using a spatula for mixing the test article with vehicle. The preparations were made immediately prior to each dosing.

READINGS AND SCORING

The following parameters were recorded:

Erythema (E)	-	0 to 4 numerical scores
Edema (O)	-	0 to 4 numerical scores
Diameter (D)	-	mm

Erythema and edema were assessed using the following numerical grading system according to Draize:

Erythema and eschar formation:

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Edema formation:

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

STUDY CONDUCT - TREATMENT PROCEDURE

PRELIMINARY STUDY

The objective of this investigation was to identify irritant test article concentrations suitable for the induction phase of the main study. In addition, a suitable non-irritant concentration of the test article, by the topical route of administration, was identified for the challenge application.

The procedure employed for these investigations was as follows:

Intradermal injections:

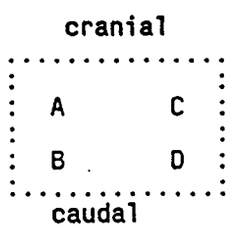
Intradermal injections (0.1 ml/site) were made into the clipped flank of two guinea-pigs at concentrations of 5, 3 and 1% of the test article in oleum arachides. The resulting dermal reactions were assessed 24 hours later.

Epidermal applications:

Patches of filter paper (2 x 2 cm) were saturated with concentrations of 5%, 3% 1% and 03% of the test article in vaseline and applied to the clipped and shaved flanks of each of four guinea-pigs. The patches were covered by a strip of aluminum foil and firmly secured by elastic plaster wrapped around the trunk and covered with impervious adhesive tape. This procedure ensured the intensive contact of the test article. The dressings were removed after an exposure period of 24 hours and the reaction sites were assessed for erythema and edema on a numerical basis according to the scale described above 24 and 48 hours after removal of the dressings.

Two previous epidermal pretests were performed as described above, one with the undiluted test article and 75%, 50% and 25% test article in vaseline, the second with 25%, 15%, 10% and 5% test article in vaseline. The third epidermal pretest described above was performed to confirm the previous results and to determine the highest non-irritating concentration.

The position of the epidermal applications is shown below:



The allocation of the different test sites on the animals was alternated in order to minimize site to site variation in responsiveness.

MAIN STUDY

Induction

Intradermal injections:

An area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped free of hair. Three pairs of intradermal injections (0.1 ml/site) were made at the border of a 4 x 6 cm area in the clipped region as follows:

Test group:

1) Freund's complete adjuvant 50:50 with bi-distilled water.

2) The test article, diluted to 5 % with oleum arachides.

3) The test article at the concentration used in (2), emulsified in a 50:50 mixture of Freund's complete adjuvant and the vehicle used in (2).

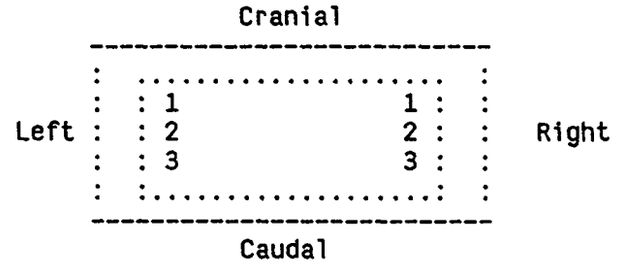
Control Group:

1) Freund's complete adjuvant 50:50 with bi-distilled water.

2) Vehicle used in (2) for test group.

3) Freund's complete adjuvant 50:50 with bi-distilled water.

The positions of the intradermal injections are shown below:



---------- clipped area
.... area in which injections were made

Epidermal applications:

One week after the injections, the scapular area (approximately 6 x 8 cm) was again clipped and shaved free of hair. A 2 x 4 cm patch of filter paper was saturated with the test article (10% in vaseline) and placed over the injection sites of the test animals. The patch was covered by aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The dressings were left in place for approximately 48 hours. The epidermal application procedure described ensured intensive contact of the test article.

The guinea-pigs of the control group were treated as described above with the omission of test article.

Reaction sites were assessed for erythema and edema 24 and 48 hours after removal of the dressing, using the numerical grading system described previously.

Challenge

The test and control guinea-pigs were challenged two weeks after the epidermal induction application.

Hair was clipped and shaved from a 5 x 5 cm area on the left and right flank of each guinea-pig. Two patches (2 x 2 cm) of filter paper were saturated with a) non-irritant concentration (1 % in vaseline) of the test article and b) with the vehicle only and applied to the (a) left flank and (b) right flank using the same method as for the epidermal application.

The dressings were removed approximately 24 hours later. The sites were assessed for erythema and edema 24 and 48 hours after removal of the dressing, using the numerical scoring system as described under preliminary study.

The control animals were treated in the same way as described above.

Erythema and edema reactions were described in the tables under Appendix A.

Re-challenge

A second challenge was performed two weeks after the first challenge.

The treatment procedure for the animals of the test group was similar as described for the first challenge with the exception that the flanks of all guinea-pigs and the vehicle used for the test article dilution were changed (a - vehicle; b - 1% test article in oleum arachides).

The control animals were treated with the vehicle alone on the left flank.

Reading of challenge reactions

The challenge site was evaluated 24 and 48 hours after removal of the patch. The readings were made under artificial fluorescent light (daylight spectrum).

Redness constitutes the minimum criterion of an allergic reaction. Strongly sensitized animals display a vivid redness, associated with indurated swelling. The reactions were scored on the basis of the Draize score described under "Readings and Scoring".

Rating of allergenicity

Based upon the percentage of animals sensitized (24-hour reading), the test article was assigned to one of the following five grades of allergenic potency, ranging from weak to extreme.

Sensitization Rate [%]	Grade	Classification
0 - 8	1	weak
9 - 28	2	mild
29 - 64	3	moderate
65 - 80	4	strong
81 - 100	5	extreme

OBSERVATIONS

In addition to the sensitizing reactions the following observations and data were recorded during the test and observation period:

Mortality/Viability	Daily
Body Weights	At acclimatization start, start of application and termination of test
Symptoms (local/ systemic)	Daily
Skin reactions	at the time of reactions readings during induction and challenge period.

Records were maintained on all additional and standard observations.

PATHOLOGY

Necropsy
No necropsy was performed in the animals euthanized at termination of observation.

All animals were euthanized at the end of the test period with an intraperitoneal injection of T61 (Hoechst AG) and discarded.

STATISTICAL ANALYSIS

Mean values with standard deviations.

Fisher-Test (The Exact Fisher Test for comparison of the basic probability of two binomial distributions. L. Sachs, Statistische Auswertungsmethoden, Georg Thieme Verlag, Stuttgart 1971).

For calculation of p-values the 24-hour reading of the animals from the control and test group was used.

DATA COMPILATION

The following data were recorded on data sheets and transcribed for compilation and analysis:

skin reactions,
mortality,
symptoms (local/systemic).

The following data were recorded on-line:

body weights.

RESULTS

Main Study

SENSITIZING EFFECTS

CONTROL GROUP:

No positive reactions were evident after the first and second challenge application, neither when treated with the vehicle alone nor when treated with the 1% test article dilution.

TEST GROUP:

First challenge:

Sixteen out of 20 animals showed positive erythema reactions after the 24-hour reading when treated with the 1% test article dilution in vaseline. Additionally positive erythema reactions were observed in 14 out of 20 animals at the 48-hour reading.

Second challenge:

One out of 20 animals showed positive erythema reactions after 24 hours when treated with the 1% test article dilution in oleum arachidis.
The second challenge was only performed to show the reactions after the use of different vehicles.

MORTALITY / VIABILITY

No death occurred during the study.

SYMPTOMS, LOCAL

CONTROL GROUP:

Application area around the injection sites 1 and 3 was found to show erythema and edema from day 2 to 7; necroses were observed from day 8 to 21 and encrustations from day 20 to 39 (termination of test). The injection site 2 showed erythema and edema from day 2 to 7.

TEST GROUP:

Application area around the injection sites 1, 2 and 3 was found to show the same local symptoms as described above for the injection sites 1 and 3 of the control group.

On day 9 of test no observation could be performed because the animals were bandaged semi-occlusively.

SYMPTOMS, SYSTEMIC

No systemic symptoms were observed in the animals.

BODY WEIGHTS

The body weight gain of the animals was not affected adversely during the study.

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

APPENDIX A

PRETEST

MAIN STUDY - INDUCTION - Epidermal Reactions
- CHALLENGES - Epidermal Reactions

PRETEST

During pretest, the following reactions were observed:

INTRADERMAL INJECTION

Vehicle: Oleum arachides

Animal No.	Sex	Concentration %	REACTION READINGS AFTER 24 HOURS		
			Erythema	Edema	Diameter [mm]
691	M	5	1	1	10 x 11
796	F	5	1	1	10 x 10
691	M	3	1	1	8 x 9
796	F	3	1	1	8 x 9
691	M	1	1	1	6 x 6
796	F	1	0	0	0

* According to Magnusson - Kligman and to the findings observed, the concentration selected for the main study was 5%.

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

PRETEST cont'd

EPIDERMAL APPLICATION I

Vehicle: Vaseline

Animal No.	Sex	Concentration [%]	REACTION READINGS AFTER REMOVAL OF BANDAGE			
			after 24 hours		after 48 hours	
			E	O	E	O
692	~~f~~ m	100	1	0	1	0
		75	1	0	2	1
		50	2	1	3	1
		25	1	0	1	0
797	f	25	1	1	1	0
		100	2	2	1	0
		75	3	1	2	0
		50	1	0	1	0
693	~~f~~ m	50	1	0	1	0
		25	1	0	1	0
		100	1	0	1	0
		75	1	0	1	0
798	f	75	1	0	0	0
		50	1	0	1	0
		25	0	0	0	0
		100	1	0	1	0

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

PRETEST cont'd

EPIDERMAL APPLICATION II

Vehicle: Vaseline

Animal No.	Sex	Concentration [%]	REACTION READINGS AFTER REMOVAL OF BANDAGE			
			after 24 hours		after 48 hours	
			E	O	E	O
493	m	25	3	2	3	2
		15	3	2	3	2
		10	2	1	2	1
		5	1	1	1	1
497	f	5	1	1	1	0
		25	2	1	1	1
		15	3	2	3	2
		10	1	1	1	1
494	m	10	1	0	0	0
		5	0	0	0	0
		25	1	0	1	1
		15	1	0	1	0
498	f	15	1	0	1	1
		10	1	0	1	1
		5	0	0	0	0
		25	1	0	1	1

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

PRETEST cont'd

EPIDERMAL APPLICATION III

Vehicle: Vaseline

Animal No.	Sex	Concentration [%]	REACTION READINGS AFTER REMOVAL OF BANDAGE			
			after 24 hours		after 48 hours	
			E	O	E	O
1	m	5	1	0	1	0
		3	1	0	0	0
		1	0	0	0	0
		0.3	0	0	0	0
2	f	0.3	0	0	0	0
		5	1	0	0	0
		3	1	0	0	0
		1	0	0	0	0
3	m	1	0	0	0	0
		0.3	0	0	0	0
		5	0	0	0	0
		3	0	0	0	0
4	f	3	0	0	0	0
		1	0	0	0	0
		0.3	0	0	0	0
		5	1	0	0	0

According to Magnusson - Kligman, and to the findings observed, the concentration selected for the induction period was 10% (see second epidermal application) and for the challenge procedure was 1%.

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - INDUCTION

TABLE 1: CONTROL GROUP
SKIN RESPONSE AFTER THE EPIDERMAL APPLICATION
OF THE VEHICLE (VASELINE) DURING INDUCTION PERIOD
(NUCHAL SKIN)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage			
		24 hours		48 hours	
		E	O	E	O
61	male	0	0	0	0
62	male	0	0	0	0
63	male	0	0	0	0
64	male	0	0	0	0
65	male	0	0	0	0
76	female	0	0	0	0
77	female	0	0	0	0
78	female	0	0	0	0
79	female	0	0	0	0
80	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN-STUDY - INDUCTION

TABLE 2: TEST GROUP
SKIN RESPONSE AFTER THE EPIDERMAL APPLICATION OF O-CRESYLGLYCIDYLETHER (CGE) (10% IN VASELINE) DURING INDUCTION PERIOD (NUCHAL SKIN)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage			
		24 hours		48 hours	
		E	O	E	O
66	male	0	0	0	0
67	male	1	0	1	0
68	male	1	0	0	0
69	male	1	0	1	0
70	male	0	0	0	0
71	male	1	0	1	0
72	male	1	0	1	0
73	male	0	0	0	0
74	male	1	0	1	0
75	male	1	0	1	0
81	female	1	0	0	0
82	female	0	0	0	0
83	female	0	0	0	0
84	female	1	0	1	0
85	female	0	0	0	0
86	female	0	0	0	0
87	female	1	0	1	0
88	female	1	0	0	0
89	female	1	0	1	0
90	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - CHALLENGE

TABLE 3: CONTROL GROUP
SKIN RESPONSE AFTER THE FIRST CHALLENGE PROCEDURE
TEST ARTICLE-TREATED, 1% IN VASELINE (LEFT FLANK)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage			
		24 hours		48 hours	
		E	O	E	O
61	male	0	0	0	0
62	male	0	0	0	0
63	male	0	0	0	0
64	male	0	0	0	0
65	male	0	0	0	0
76	female	0	0	0	0
77	female	0	0	0	0
78	female	0	0	0	0
79	female	0	0	0	0
80	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - CHALLENGE

TABLE 4: CONTROL GROUP
SKIN RESPONSE AFTER THE FIRST CHALLENGE PROCEDURE
VEHICLE, VASELINE (RIGHT FLANK)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage			
		24 hours		48 hours	
		E	O	E	O
61	male	0	0	0	0
62	male	0	0	0	0
63	male	0	0	0	0
64	male	0	0	0	0
65	male	0	0	0	0
76	female	0	0	0	0
77	female	0	0	0	0
78	female	0	0	0	0
79	female	0	0	0	0
80	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - CHALLENGE

TABLE 5: TEST GROUP
SKIN RESPONSE AFTER THE FIRST CHALLENGE PROCEDURE
TEST ARTICLE-TREATED, 1% IN VASELINE (LEFT FLANK)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage 24 hours		48 hours	
		E	O	E	O
66	male	0	0	0	0
67	male	0	0	0	0
68	male	1	0	0	0
69	male	1	0	1	0
70	male	1	1	1	0
71	male	2	1	1	0
72	male	1	1	1	0
73	male	0	0	0	0
74	male	2	1	1	0
75	male	1	0	1	0
81	female	1	0	1	0
82	female	1	0	1	0
83	female	1	0	1	0
84	female	2	1	1	0
85	female	2	1	1	0
86	female	1	0	0	0
87	female	1	0	1	0
88	female	0	0	0	0
89	female	1	0	1	0
90	female	1	0	1	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - CHALLENGE

TABLE 6: TEST GROUP
SKIN RESPONSE AFTER THE FIRST CHALLENGE PROCEDURE
VEHICLE, VASELINE (RIGHT FLANK)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage 24 hours		48 hours	
		E	O	E	O
66	male	0	0	0	0
67	male	0	0	0	0
68	male	0	0	0	0
69	male	0	0	0	0
70	male	0	0	0	0
71	male	0	0	0	0
72	male	0	0	0	0
73	male	0	0	0	0
74	male	0	0	0	0
75	male	0	0	0	0
81	female	0	0	0	0
82	female	0	0	0	0
83	female	0	0	0	0
84	female	0	0	0	0
85	female	0	0	0	0
86	female	0	0	0	0
87	female	0	0	0	0
88	female	0	0	0	0
89	female	0	0	0	0
90	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - CHALLENGE

TABLE 7: CONTROL GROUP
SKIN RESPONSE AFTER THE SECOND CHALLENGE PROCEDURE
VEHICLE, OLEUM ARACHIDES (LEFT FLANK)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage			
		24 hours		48 hours	
		E	O	E	O
61	male	0	0	0	0
62	male	0	0	0	0
63	male	0	0	0	0
64	male	0	0	0	0
65	male	0	0	0	0
76	female	0	0	0	0
77	female	0	0	0	0
78	female	0	0	0	0
79	female	0	0	0	0
80	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - CHALLENGE

TABLE 8: TEST GROUP
SKIN RESPONSE AFTER THE SECOND CHALLENGE PROCEDURE
TEST ARTICLE-TREATED, 1% IN OLEUM ARACHIDES (RIGHT FLANK)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage			
		24 hours		48 hours	
		E	O	E	O
66	male	0	0	0	0
67	male	0	0	0	0
68	male	0	0	0	0
69	male	0	0	0	0
70	male	0	0	0	0
71	male	1	0	0	0
72	male	0	0	0	0
73	male	0	0	0	0
74	male	0	0	0	0
75	male	0	0	0	0
81	female	0	0	0	0
82	female	0	0	0	0
83	female	0	0	0	0
84	female	0	0	0	0
85	female	0	0	0	0
86	female	0	0	0	0
87	female	0	0	0	0
88	female	0	0	0	0
89	female	0	0	0	0
90	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

MAIN STUDY - CHALLENGE

TABLE 9: TEST GROUP
SKIN RESPONSE AFTER THE SECOND CHALLENGE PROCEDURE
VEHICLE, OLEUM ARACHIDES (LEFT FLANK)

Animal Number	Sex	Erythema/Edema-readings after removal of bandage			
		24 hours		48 hours	
		E	O	E	O
66	male	0	0	0	0
67	male	0	0	0	0
68	male	0	0	0	0
69	male	0	0	0	0
70	male	0	0	0	0
71	male	0	0	0	0
72	male	0	0	0	0
73	male	0	0	0	0
74	male	0	0	0	0
75	male	0	0	0	0
81	female	0	0	0	0
82	female	0	0	0	0
83	female	0	0	0	0
84	female	0	0	0	0
85	female	0	0	0	0
86	female	0	0	0	0
87	female	0	0	0	0
88	female	0	0	0	0
89	female	0	0	0	0
90	female	0	0	0	0

E = Erythema
O = Oedema

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

APPENDIX B

BODY WEIGHTS

BODY WEIGHTS (GRAM) SUMMARY
MALES

ACCLIMATIZATION		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP
DAY 1	MEAN	336.0	327.6
WEEK 1	ST.DEV.	15.2	15.9
	N	5	10

BODY WEIGHTS (GRAM) SUMMARY
MALES

TREATMENT			GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP
DAY	1	MEAN	399.2	386.9
WEEK	1	ST.DEV.	14.1	17.2
		N	5	10
DAY	39	MEAN	638.3	625.4
WEEK	6	ST.DEV.	44.9	42.3
		N	5	10

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

ACCLIMATIZATION		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP
DAY 1	MEAN	332.1	335.4
WEEK 1	ST.DEV.	5.1	5.8
	N	5	10

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP
DAY 1	MEAN	391.9	384.4
WEEK 1	ST.DEV.	23.6	20.6
	N	5	10
DAY 39	MEAN	623.1	566.9
WEEK 6	ST.DEV.	52.5	75.7
	N	5	10

BODY WEIGHTS (GRAM)
MALES

GROUP 1 (CONTROL GROUP)

	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	39
WEEKS	1	1	6
ANIMAL			
61	348.5	419.3	623.2
62	344.3	384.2	604.2
63	331.4	395.8	600.1
64	311.4	389.4	708.5
65	344.5	407.1	655.4

GROUP 2 (TEST GROUP)

	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	39
WEEKS	1	1	6
ANIMAL			
66	350.4	385.7	583.2
67	315.4	363.6	599.1
68	343.1	390.7	607.5
69	347.9	418.1	655.1
70	308.4	385.4	681.5
71	327.4	381.0	651.2
72	327.5	370.3	645.7
73	334.1	410.9	628.2
74	310.1	391.4	660.5
75	312.0	371.7	541.9

BODY WEIGHTS (GRAM)
FEMALES

GROUP 1 (CONTROL GROUP)

	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	39
WEEKS	1	1	6
ANIMAL			
76	328.6	353.8	532.4
77	337.5	397.1	648.6
78	329.7	391.1	639.6
79	337.7	418.5	666.1
80	327.0	399.0	628.6

GROUP 2 (TEST GROUP)

	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	39
WEEKS	1	1	6
ANIMAL			
81	340.4	348.9	411.2
82	331.3	409.1	659.9
83	333.4	398.6	619.7
84	338.5	376.1	487.9
85	339.9	412.8	536.3
86	323.9	357.6	563.1
87	333.1	388.7	653.8
88	344.7	392.9	596.3
89	335.0	378.1	559.6
90	334.3	381.4	580.8

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

APPENDIX C

EXACT FISHER TEST

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

SENSIBILIZATION TEST

FIRST CHALLENGE (1 % in VASELINE)

Reading after 24 hours:

1. Test pair: Control Group

Positive : 0

Negative : 10

2. Test pair: Test-Group

Positive : 16

Negative : 4

EXACT FISHER-TEST PROBABILITY (ONE SIDED): < 0.001

SECOND CHALLENGE (1% in OLEUM ARACHIDIS)

Reading after 24 hours:

1. Test pair: Control Group

Positive : 0

Negative : 10

2. Test pair: Test-Group

Positive : 1

Negative : 19

EXACT FISHER-TEST PROBABILITY (ONE SIDED): 0.667

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

APPENDIX D

WATER ANALYSES -

BACTERIOLOGICAL AND CHEMICAL ASSAYS

CONTAMINANT ANALYSIS OF DRINKING WATER

CONTAMINANT ANALYSES OF FEED

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

BACTERIOLOGICAL ASSAY OF DRINKING WATER, ITINGEN

Official Laboratory
Basel-Landschaft

Liestal, 14.06.90
Ref.no. 90060086
90060090

Sampling points:	1) 59.1.AU, source water, UV-irradiated 2) 59.A.1, Pumping station "Gstaadmatt"	
Sampled on:	11.06.90	
Sample:	1)	2)
Time of Sampling	07.45	08.15
Water temperature (°C)	8.7	11.3
Air temperature (°C)	19.0	19.0
Weather condition prior to sampling	rain	
Weather condition during sampling	cloudy	

BACTERIOLOGICAL TEST:

Aerobic mesophilic bacteria /ml	4	11
E.coli /100 ml	0	0
Enterococci /100 ml	0	0

ASSESSMENT:

At the time of sampling, the tested bacteriological parameters were flawless, and met the requirements for drinking water according to article 260 of the "Eidg. Lebensmittelverordnung".

Official Laboratory
The Official Chemist
(signed Dr. W. Stutz)

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

BACTERIOLOGICAL ASSAY OF DRINKING WATER, ITINGEN

Official Laboratory
Basel-Landschaft

Liestal, 24.09.90
Ref.no. 90090138
90090139
90090141

Sampling points: 1) 59.1.AU, source water, UV-irradiated
2) 59.70.N, water from Sissach
3) 59.A.1, Pumping station "Gstaadmatt"

Sampled on: 13.09.90

Sample:	1)	2)	3)
Time of Sampling	08.00	08.30	09.00
Water temperature (°C)	9.8	14.9	11.4
Air temperature (°C)	15.0	15.0	15.0

Weather condition prior to sampling: sunny

Weather condition during sampling: sunny

BACTERIOLOGICAL TEST:

Aerobic mesophilic bacteria /ml	173	5	5
E.coli /100 ml	0	0	0
Enterococci /100 ml	0	0	0
Nitrate			23.5
Chloride			20.7

ASSESSMENT:

At the time of sampling, the tested bacteriological parameters were flawless, and met the requirements for drinking water according to article 260 of the "Eidg. Lebensmittelverordnung".

Official Laboratory
The Official Chemist
(signed Dr. W. Stutz)

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

CHEMICAL WATER ANALYSIS, ITINGEN

Official Laboratory
Basel-Landschaft

Liestal, 14.06.90
Ref.No. 90060086

Location: 59.A.1, Pumping station "Gstaadmatt"

Date of sampling	11.06.90
Time of sampling	08.15 a.m.
Water temperature (°C)	11.3
Air temperature (°C)	19.0
Weather condition prior to sampling	rain
Weather condition during sampling	cloudy
Appearance	clear, colorless
Odor	not remarkable
Taste	not remarkable

Total hardness		fr.H°	41.8
Alkaline hardness		fr.H°	28.0
Non carbonate hardness		fr.H°	13.8
Conductivity at 20 °C		µS/cm	655.0
Oxygen demand (KMnO4 cons.)		mg/l	2.1
Free ammonia	NH_4^+	mg/l	<0.0100
Nitrite	NO_2^-	mg/l	<0.0050
Nitrate	NO_3^-	mg/l	21.7
Chloride	Cl^-	mg/l	19.8
Sulphate	SO_4^{--}	mg/l	112.8
Calcium	Ca^{++}	mg/l	145.5
Magnesium	Mg^{++}	mg/l	13.2
Dry residue		mg/l	588
pH electrometrically			7.12
Free carbonic acid CO_2 potentiometric			35.0
Free carbonic acid CO_2		mg/l	52.3
Excess carbonic acid CO_2		mg/l	0.0
Oxygen		mg/l	6.74
Oxygen % saturation			62.4
Phosphate		mg/l	<0.005

The water sample meets the chemical requirements for drinking water according to article 260 of the "Eidg. Lebensmittelverordnung". The values for nitrate and chloride exceed the recommended limits slightly.

Official Laboratory
The Official Chemist
(signed Dr. W. Stutz)

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

CONTAMINANT ANALYSIS OF DRINKING WATER, ITINGEN

RCC Project: 278010

Date of sampling: 27.06.90

Sample: Tap water RCC Itingen, Room U 10

Parameter	Assay level mg/kg
Lindane	<0.005
Heptachlor	<0.005
Malathion	<0.5
DDT, total	<0.025
Dieldrin	<0.005
PCB's	<0.025
Cadmium	<0.02
Arsenic	<0.15
Lead	<0.25
Mercury	<0.05
Selenium	<0.15
Copper	<0.15
Nitrosamines (DMN, DEN, NPIP, NMORPH), total	<0.01

< 0.001 = less than 0.001 milligram per kilogram

July 11, 1990
signed K.Biedermann

RCC

Umweltchemie AG

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

ANALYTICAL TEST REPORT

Project 278785
10.07.1990

Prepared for	: Klingentalmühle AG CH-4303 Kaiseraugst
Attention of	: Dr. A. Oharek
Materials tested	: Kliba 342, Batch 59/90 05.07.1990
Tests performed	: AAS, GC, GC-MS, HPLC
Test results	: See attached Table 1
Submitted	: J. Walker
Issued by	: K. Biedermann

F. Biedermann

July 24, 1990 /sad

The undersigned confirms that analysis of KLIBA-feed (number 342, Batch 59/90, manufactured 05.07.1990) was performed, and that this certificate represents accurately the analysis results of the feed delivered.

Date: 25.07.1990

KLINGENTALMUEHLE AG

RCC

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

Attachment

Project 278785
10.07.1990

Table 1 - Test Results Kliba 342, Batch 59/90
05.07.1990

Parameter	Assay level mg/kg
Aflatoxins (B1, B2, G1, G2), total	<0.001
Estrogens (DES, Hexestrol, Dienestrol), total	<0.001
Lindane	<0.005
Heptachlor	<0.005
Malathion	<0.5
DDT, total	<0.025
Dieldrin	<0.005
Cadmium	0.06
Arsenic	<0.15
Lead	0.69
Mercury	<0.05
Selenium	0.23
Copper	15
PCBs	<0.025
Nitrosamines (DMN, DEN, NPIP, NMORPH), total	<0.01

<0.001 = less than 0.001 milligram per kilogram

The original certificate of analysis has been archived by KLIBA of Kaiseraugst.

Date: 25.07.1990 KLINGENTALMUEHLE AG

RCC

Umweltchemie AG

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

ANALYTICAL TEST REPORT

Project 284275
19.09.1990

Prepared for	: Klingentalmühle AG CH-4303 Kaiseraugst
Attention of	: Dr. A. Oharek
Materials tested	: Kliba 342, Batch 60/90 18.09.1990
Tests performed	: AAS, GC, GC-MS, HPLC
Test results	: See attached Table 1
Submitted	: J. Walker
Issued by	: K. Biedermann

K. Biedermann
October 03, 1990/sad

The undersigned confirms that analysis of KLIBA-feed (number 342, Batch 60/90, manufactured 18.09.1990) was performed, and that this certificate represents accurately the analysis results of the feed delivered.

Date: 04.10.1990

KLINGENTALMUEHLE AG

CH-4452 Itingen/Basel Switzerland Telephone 061/98 52 52 Telex 966156 rcc ch Telefax 061/98 52 66

RCC

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

Attachment

Project 284275
19.09.1990

Table 1 - Test Results Kliba 342, Batch 60/90
19.09.1990

Parameter	Assay level mg/kg
Aflatoxins (B1, B2, G1, G2), total	<0.001
Estrogens (DES, Hexestrol, Dienestrol), total	<0.001
Lindane	<0.005
Heptachlor	<0.005
Malathion	<0.5
DDT, total	<0.025
Dieldrin	<0.005
Cadmium	0.07
Arsenic	<0.15
Lead	1.13
Mercury	<0.05
Selenium	<0.15
Copper	19
PCBs	<0.025
Nitrosamines (DMN, DEN, NPIP, NMORPH), total	<0.01

<0.001 = less than 0.001 milligram per kilogram

The original certificate of analysis has been archived by KLIBA of Kaiseraugst.

Date: 04.10.1990

KLINGENTALMUEHLE AG

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

APPENDIX E

REFERENCE VALUES, POSITIVE CONTROL

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

PROJECT 270066

Test for contact hypersensitivity in the albino guinea pig with FORMALDEHYDLOESUNG (HCHO)
The guinea pig maximization test

Positive Control

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

SUMMARY AND CONCLUSION

"Allergic Contact Dermatitis in the Guinea Pig: Identification of Contact Allergens" Magnusson B. Kligman A. M., 1970 published by C. C. Thomas, Springfield, Illinois, U. S. A.

According to the procedures used in this experiment (run from April 23 to May 24, 1990), clear positive results were observed in the HCHO treated animals after the epidermal challenge application.

POSITIVE ERYTHEMA REACTIONS AFTER FIRST CHALLENGE PROCEDURE

	after 24 hours positive / total % positive out of total
POSITIVE CONTROL HCHO	7 / 10 70

For the induction period a 20 % dilution of HCHO in bi-distilled water and for the challenge procedure a 15 % dilution of HCHO was used.

According to the results observed it is considered that HCHO possess an strong skin sensitizing (contact allergenic) potential in the guinea pig strain used (Ibm: GOHI; SPF-quality guinea pigs (synonym: Himalayan spotted); BRL, Biological Research Laboratories Ltd., CH-4414 Füllinsdorf)

The positive control article FORMALDEHYDLOESUNG (HCHO) was delivered by Fluka AG, 9470 Buchs, Switzerland (Article No. 4003), and the purity was described to be at least 37%.

The raw data from this project are kept in a separate file at RCC. The test described above was performed under GLP-conditions with a QA-check.

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

SKIN RESPONSE AFTER FIRST CHALLENGE PROCEDURE

Positive Control - HCHO

Animal Number/Sex	Erythema-readings after removal of bandage		
	immediately	24 hours	48 hours
103 m	1	1	0
104 m	0	0	0
105 m	2	1	1
106 m	2	1	1
107 m	1	0	0
111 f	1	1	1
112 f	1	1	0
113 f	0	0	0
114 f	2	1	0
115 f	2	1	0

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

APPENDIX F

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

Test article identification: Name: O-CRESYLGLYCIDYLETHER (CGE)				SUMMARY TABLE
Lot Batch No: DC 1294.1				
SKIN TOLERANCE STUDIES / IMMUNOSTIMULATION (Sensitization potential by intradermal and epicutaneous administration) Maximization Test (MT)				Study No: 272913 Report date: Jan. 25, 1991
Species/Strain: Himalayan white spotted GP				Number of exp. animals: 44
Procedure	Administration route/site		Day	Vehicle
First induction	intradermal/suprascapular		1	1. FCA:bi-dist.water (1:1) 2. OLEUM ARACHIDIS 3. FCA:OL. ARA. (1;1) VASELINE
Second induction	occl. patch/suprascapular		8	
Challenge I	occl. patch/left flank		22	OLEUM ARACHIDIS
Study group	Control group		Test group	
	Conc. of test art. in %	No. of appl. and dose	Conc. of test art. in %	No. of appl. and dose
First induction	vehicle	4x100µl/i.d.	5	4x100µl/i.d.
Second induction	vehicle	saturated patch/8cm²	10	saturated patch/8cm²
Challenge I A	1	saturated patch/4cm²	1	saturated patch/4cm²
B	vehicle	saturated patch/4cm²	vehicle	saturated patch/4cm²
Sex	f/m		f/m	
Number of animals	5/5		10/10	
Animals with pos. react. — Chall.(24h) (48h after appl.) A	0/10		16/20	
B	0/10		0/20	
Chall.(48h) (72h after appl.) A	0/10		14/20	
B	0/10		0/20	
Summary of salient findings: Strong allergenic potency of the test article after the first challenge.				
Study conducted by the applicant: yes < > no <X> Study in compliance with GLP: yes <X> no < > QAU inspected: yes <X> no < >				

* Informations concerning the second challenge are summarized on the following page.

RCC PROJECT 272913
O-CRESYLGLYCIDYLETHER (CGE)

Test article identification: Name: O-CRESYLGLYCIDYLETHER (CGE)				SUMMARY TABLE	
Lot Batch No: DC 1294.1					
SKIN TOLERANCE STUDIES / IMMUNOSTIMULATION (Sensitization potential by intradermal and epicutaneous administration) Maximization Test (MT)				Study No: 272913 Report date: Jan. 25, 1991	
Species/Strain: Himalayan white spotted GP				Number of exp. animals: 44	
Procedure		Administration route/site		Day	Vehicle
First induction		intradermal/suprascapular		1	1. FCA:bi-dist.water (1:1) 2. OLEUM ARACHIDIS 3. FCA:OL. ARA. (1;1) VASELINE
Second induction		occl. patch/suprascapular		8	
Challenge II		occl. patch/left flank		36	OLEUM ARACHIDIS
Study group		Control group		Test group	
		Conc. of test art. in %	No. of appl. and dose	Conc. of test art. in %	No. of appl. and dose
First induction		vehicle	4x100µl/i.d.	5	4x100µl/i.d.
Second induction		vehicle	saturated patch/8cm²	10	saturated patch/8cm²
Challenge II	A	1	saturated patch/4cm²	1	saturated patch/4cm²
	B	vehicle	saturated patch/4cm²	vehicle	saturated patch/4cm²
Sex		f/m		f/m	
Number of animals		5/5		10/10	
Animals with pos. react.	Chall.(24h) (48h after appl.) A	0/10		1/20	
	B	0/10		0/20	
	Chall.(48h) (72h after appl.) A	0/10		0/20	
	B	0/10		0/20	
Summary of salient findings: Weak allergenic potency of the test article after the second challenge.					
Study conducted by the applicant: yes < > no <X> Study in compliance with GLP: yes <X> no < > QAU inspected: yes <X> no < >					

GROUP RESEARCH REPORT

SBGR.92.257

500 70 649 SP 4904

Monobutyl-p-cresol: Bacterial mutagenicity studies

[redacted]

SICC, CTMS

2

[redacted] SBGR.92.257

Study Title

Monobutyl-p-cresol: Bacterial mutagenicity studies

Regulatory Data Requirement

Directive 67/548/EEC, Sixth Amendment

Authors

[redacted]

Study Completed On

11th February 1993

Performing Laboratory

Sittingbourne Research Centre,
Sittingbourne, Kent, ME9 8AG, England

Laboratory Project Identity

Experiment No. 5765 Report No. SBGR.92.257

(Total number of pages in the study: 47)

This page is reserved for information relevant to regulatory submission.

COMPLIANCE WITH GOOD LABORATORY PRACTICE

This study has been conducted in compliance with GOOD LABORATORY PRACTICE and meets the following requirements:

United States Environmental Protection Agency 40 CFR 160

United Kingdom Department of Health, Principles of Good Laboratory Practice LONDON 1989

Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice PARIS 1982

Japan Ministry of Agriculture Forestry and Fisheries
59 NohSan Notification No. 3850 1984

except that no claim of GLP compliance is made in respect of the data for the characterisation of the test substance reported.

This report fully and accurately reflects the raw data generated in the study.

Study Director: [redacted]
(Signature)

11/2/93
(Date)

QUALITY ASSURANCE STATEMENT

REPORT NUMBER: SBGR.92.257

EXPERIMENT NUMBER: 5765

REPORT TITLE: Monobutyl-p-cresol: Bacterial mutagenicity studies

STUDY DIRECTOR: [redacted]

The conduct of this study was inspected on the dates given below. In addition, routine procedures carried out in all studies of this type have been inspected at intervals according to a predetermined schedule and the relevant dates are also given below.

This report has been audited to ensure that it accurately describes the methods used and that the reported results accurately reflect the raw data of the study.

Date of inspection or audit	Date of QA report to Management
8.10.92	8.10.92
14-16.10.92	16.10.92
20.10.92	20.10.92
9. 2.93	9. 2.93

[redacted]

22nd. February 1993

[redacted]
QUALITY ASSURANCE UNIT

[redacted] SBGR.92.257

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
Shell Research Limited
Sittingbourne Research Centre
Sittingbourne
Kent
ME9 8AG

DATE OF INSPECTION

17 August 1992

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities.

D.F. Moore

30/10/92 D. F. Moore
Director
UK GLP Monitoring Unit

CHRONOLOGY OF STUDY

Approval of Protocol	16th October 1992
Protocol Amendment	21st October 1992
Commencement of bacterial mutagenicity assays	22nd October 1992
Completion of bacterial mutagenicity assays	12th November 1992

LOCATION of Raw Data, Specimens and Final Report:

Sittingbourne Research Centre
Sittingbourne ME9 8AG
England

[redacted]
SBGR.92.257

Monobutyl-p-cresol: Bacterial mutagenicity studies

(Experiment Number 5765)

SUMMARY:

The mutagenic activity of monobutyl-p-cresol was investigated in agar layer cultures of selected bacterial tester strains of Salmonella typhimurium and Escherichia coli. Assays were performed both in the presence and in the absence of an S9 microsomal fraction obtained from a liver homogenate from rats pre-treated with Aroclor 1254.

It was concluded that monobutyl-p-cresol did not induce reverse gene mutation in the selected bacterial tester strains, under the experimental conditions described.

[redacted]

pp [redacted], Ph.D., Manager & Director Research,
Shell Research Limited,
Sittingbourne Research Centre,
Sittingbourne, Kent, ME9 8AG, England.

Date: February 22nd 1993

TEXT:

CONTENTS

PROFESSIONAL AND SUPERVISORY PERSONNEL INVOLVED IN STUDY

Dr. D.E. Wiggins	Formulation Chemist and Compound Controller
Dr. T.M. Brooks	Study Director

Scientific Reviewer: J. Hooson, B.Sc., Ph.D.

STUDY DIRECTOR: [redacted]., Ph.D.

[redacted] 11/2/93

(Signature) (Date)

INTRODUCTION

This report describes the results from short-term bacterial mutagenicity assays designed to investigate the genotoxicity of monobutyl-p-cresol.

These assays determine the effect of monobutyl-p-cresol on reverse gene mutation in selected bacterial tester strains.

MATERIALS AND METHODS

1. Bacteria

The Salmonella typhimurium strains[1] TA98, TA100, TA1535, TA1537 and TA1538 were obtained from Dr. B.N. Ames, University of California, Berkeley, California, USA.

Escherichia coli WP_2 uvrA pKM101[2] was obtained from Dr. S. Venitt, current address, Institute of Cancer Research, Sutton, UK.

The genotypic characteristics of the bacterial tester strains used in this study were checked on 9th October 1992.

2. Culture media

For detecting revertants of both the Salmonella and Escherichia tester strains, ready-poured petri plates containing 25 ml of a minimal agar medium, based on Vogel and Bonner[3], were obtained from Becton Dickinson Ltd., Cowley, Oxford. Batch numbers used in this study were 0517122124, 0517122147, 0517122205 and 0517122206.

3. Chemicals

3.1 Test substance

Monobutyl-p-cresol was obtained from Derfesa, Spain. It was prepared for use as a solution in DMSO. Details of the test substance are given in Appendix 2.

3.2 Positive control compounds

The positive control compounds benzo(a)pyrene, potassium dichromate, neutral red, sodium azide, 2-nitrofluorene, 2-aminoanthracene and 9-aminoacridine, and their formulations, are described in Appendix 2.

4. Plate incorporation assay

The method used was basically that described by Maron and Ames[4], but included a pre-incubation period of bacteria, test compound and either S9 microsomal fraction obtained from a liver homogenate from male Fischer 344 rats pre-treated with Aroclor, or pH 7.4 buffer, as appropriate, before incorporation into the top agar. For all microbial assays, a final concentration of 10% S9 in the S9 mix was used. The S9 fraction used in the mutagenicity assays in this study (Batch No. 38) was prepared on 28th October 1992. The S9 mix contained per ml : S9 fraction (0.1 ml), $MgCl_2$ (8 µmol), KCl (33 µmol), G-6-P (5 µmol), NADP (4 µmol) and sodium phosphate buffer, pH 7.4 (100 µmol).

A preliminary cytotoxicity assay was first carried out to assess the cytotoxicity of the test material, its solubility in the top agar and for any effect on the pH of the test system. The amounts to be used in the mutation assays were selected on this basis with a range of doses initially up to a maximum of 50 µg per ml in the absence of S9 mix and 500 µg per ml in the presence of S9 mix for the first set of experiments. These doses were altered in some cases in subsequent experiments to take into account cytotoxicity.

In each of the bacterial mutation assays control plates were set up with the solvent alone and with an appropriate known positive control compound. All tests were carried out in triplicate. Assays were carried out on different days in order to confirm the reproducibility of the results and/or alter the dose range to take into account cytotoxic effects.

RESULTS AND DISCUSSION

The test compound, monobutyl-p-cresol, was formulated as a solution in DMSO for use in these microbial assays. These formulations were adjudged stable for at least one working day (Appendix 2). Preliminary pH checks of the media used in the bacterial assays at the maximum amounts to be tested showed no significant effects. Although addition of the test compound made the top agar appear milky there was no evidence of precipitation at any treatment level.

Because of the volatile nature of the test compound, mutagenicity assays were performed by the pre-incubation method[5] in sealed containers.

The addition of monobutyl-p-cresol up to cytotoxic concentrations did not increase reverse gene mutation in Escherichia coli WP_2 uvrA pKM101, Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 or TA 100, either in the presence or absence of rat liver S9 fraction. Evidence of cytotoxicity was observed in these assays in all bacterial tester strains. The test compound was more cytotoxic in the absence of S9 mix.

CONCLUSIONS

The results show that monobutyl-p-cresol did not induce reverse gene mutation in the selected bacterial tester strains, under the experimental conditions described.

REFERE

1.

2.

3.

4.

5.

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Published by Elsevier, New York.

[redacted]SBGR.92.257

APPENDIX 1

Microbiology Report

Title:	Monobutyl-p-cresol: Bacterial mutagenicity studies
Experiment No:	5765
Responsible Practitioner:	T.M. Brooks
Participants:	L.P. Gonzalez and V.M. Warwick
Summary of Work Done:	The mutagenic activity of monobutyl-p-cresol was investigated in agar layer cultures of Escherichia coli WP_2 uvrA pKM101, Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 or TA 100 both in the presence and in the absence of a rat liver microsomal activation system (S9).

METHODS

Because of the volatile nature of the test compound, the method used included a period of incubation of the test compound, bacteria, and S9 mix or pH 7.4 buffer, as appropriate, before incorporation into the top agar.

For the assay the following were added to sterile 30 ml glass containers: 0.5 ml of bacterial suspension, 0.1 ml of the test compound or solvent and 2.4 ml of the S9 mix or pH 7.4 buffer. The containers were then incubated in a shaking water bath at 37°C for 30 min. After incubation 0.5 ml was removed and added to 2 ml top agar. The contents were mixed, poured onto minimal agar plates, allowed to gel and the plates were incubated in sealed containers at 37°C for 48-72 hours.

100 µl volumes of solutions of monobutyl-p-cresol in DMSO (0.011, 0.023, 0.046, 0.093, 0.187, 0.375, 0.75, 1.5 or 3 mg per ml) were added to the pre-incubation mix to give final concentrations of 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 or 100 µg per ml in the absence of S9 mix or 100 µl of monobutyl-p-cresol in DMSO (0.029, 0.058, 0.117, 0.234, 0.468, 0.937, 1.875, 3.75, 7.5 or 15 mg per ml) to give final concentrations of 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 or 500 µg per ml in the presence of rat liver S9 mix.

RESULTS

A summary of the mutagenicity data is given in Table 1 and the raw data in Table 2.

Although the test compound gave a milky appearance when added to the top agar there was no evidence of precipitation at any treatment level.

The addition of monobutyl-p-cresol at a final concentration of 2400 µg per ml caused the pH of the medium to change from 7.2 to 7.4.

Microscopical evaluation of the background lawn showed evidence of cytotoxicity in all bacterial tester strains; this was greater in the absence of S9 mix.

The addition of monobutyl-p-cresol to agar layer cultures of Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 or TA 100 or Escherichia coli WP_2 uvrA pKM101 did not increase the reverse mutation frequency in any of the strains either in the presence or in the absence of rat liver S9 fraction.

The activity of the S9 mix and the sensitivities of the bacterial tester strains were monitored by treating cultures with the following known positive control compounds:

Bacterial strain	-S9	+S9
E.coli WP_2 uvrA pKM101	Potassium dichromate	Benzo(a)pyrene
S.typhimurium TA1535	Sodium azide	2-Aminoanthracene
S.typhimurium TA1537	9-Aminoacridine	Neutral Red
S.typhimurium TA1538	2-Nitrofluorene	Benzo(a)pyrene
S.typhimurium TA98	2-Nitrofluorene	Benzo(a)pyrene
S.typhimurium TA100	Sodium azide	Benzo(a)pyrene

[redacted] M.I.Biol.,Ph.D.
Responsible Practitioner.
Date: 11/2/93

Table 1 - Relative reverse mutation rates[1] in Escherichia coli WP_2 uvrA pKM101, Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 or TA 100 after treatment with monobutyl-p-cresol in the pre-incubation assay

Micro-organisms	Date of assay	Without microsomal activation: Monobutyl-p-cresol (μg/ml) 0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	(a) NaN_3 1 or 2.5 μg	(b) AAC 25 μg	(c) PD 10 μg	(d) NF 2.5 μg	With microsomal activation: Monobutyl-p-cresol (μg/ml) 0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	(e) BP 5 μg	(f) AAN 25 μg	(g) NR 10 μg
E.coli	30-10-92	1.1	0.9	1.0	1.1	1.1	1.1	1.0	1.1	-	-	-	2.8*	-	-	-	1.0	1.1	1.0	1.0	1.0	0.9	0.8	0.1+	3.6*	-	-
WP_2 uvrA pKM101	10-11-92	-	1.0	1.1	1.1	1.1	1.1	1.1	0.7	0+	-	-	5.8*	-	-	-	0.9	1.0	0.9	1.0	1.0	0.8	0.7	0.1+	7.1*	-	-
S.typhimurium	30-10-92	0.9	1.2	1.1	0.9	1.2	0.9	1.3	1.3	-	32.2*	-	-	-	-	-	0.9	1.0	1.1	0.8	0.8+	0.9+	0+	0+	-	9.9*	-
TA 1535	04-11-92	-	0.9	1.1	0.8	1.1	0.9	0.9	0.1+	0+	55.3*	-	-	-	0.8	0.9	0.7	0.9	0.7	0.9	0.6	0.8+	-	-	-	14.9*	-
S.typhimurium	30-10-92	0.8	1.0	0.9	0.8	0.7	0.6	0.6	0.3+	-	-	50.3*	-	-	-	-	0.7	0.8	0.7	0.7	0.6	0.5+	0+	0+	-	-	48.6*
TA 1537	04-11-92	-	0.7	0.7	0.7	0.7	0.7	0.9	0+	0+	-	10.5*	-	-	1.0	0.7	0.9	1.0	0.7	0.8	0.7	0.3+	-	-	-	-	17.6*
S.typhimurium	04-11-92	-	0.9	1.2	0.9	1.2	1.0	0.8	0.7+	0+	-	-	-	35.6*	1.2	1.0	0.9	1.0	1.3	1.1	0.7+	0+	-	-	18.9*	-	-
TA 1538	05-11-92	-	0.7	0.8	0.8	1.0	0.9	0.8	0.4+	0+	-	-	-	22.5*	1.0	0.8	1.1	1.1	1.3	1.1	0.5+	0+	-	-	16.5*	-	-
S.typhimurium	30-10-92	1.2	1.0	1.3	1.4	1.2	1.0	1.0	0.2+	-	-	-	-	21.4*	-	-	1.0	0.6	1.0	1.0+	0.5+	0.4+	0.7+	0.6+	13.3*	-	-
TA 98	04-11-92	-	0.8	1.0	0.9	0.8	0.8	0.7	0.4+	0+	-	-	-	20.7*	1.3	1.3	1.5	1.2	1.0	1.0+	1.4+	1.2+	-	-	21.0*	-	-
S.typhimurium	30-10-92	1.1	1.0	1.0	1.0	1.1	1.1	0.9	0.8+	-	5.9*	-	-	-	-	-	1.0	1.0	0.9	1.0	1.0	0.9	0.1	0+	3.3*	-	-
TA 100	04-11-92	-	1.0	1.1	1.0	1.2	1.2	1.0	1.0	0+	3.8*	-	-	-	-	-	0.9	1.1	1.0	1.2	1.4	1.4	1.1	0.1+	3.1*	-	-

(1) Results are expressed as a ratio: Mean number of revertant colonies per treated plate / Mean number of revertant colonies per control plate

Reproducible dose-related increases or values of 2.5 x control values or greater* are considered to indicate a mutagenic response.

- Not tested + Cytotoxic

(a) Sodium azide
(b) 9-Aminoacridine
(c) Potassium dichromate
(d) 2-Nitrofluorene
(e) Benzo(a)pyrene
(f) 2-Aminoanthracene
(g) Neutral red

Table 2 - Reverse mutation rates in S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538 and E. coli WP_2 uvrA pKM101 after treatment with monobutyl-p-cresol, benzo(a)pyrene (BP), potassium dichromate (PD), neutral red (NR), sodium azide (NaN_3), 2-nitrofluorene (NF), 2-aminoanthracene (AAN) or 9-aminoacridine (AAC) in the pre-incubation assay

The following tables show the individual plate counts; mean counts, standard deviations and the relative reverse mutation rates (TM/CM) for each bacterial tester strain in the presence and absence of rat liver S9 fraction (+S9 or -S9).

Keywords

$$TM/CM = \frac{\text{Mean number of revertants per treated plate}}{\text{Mean number of revertants per control plate}}$$

**** = no count

Positive control compounds

5 BP	= 5 µg per ml benzo(a)pyrene
10 PD	= 10 µg per ml potassium dichromate
10 NR	= 10 µg per ml neutral red
1 or 2.5 NaN_3	= 1 or 2.5 µg per ml sodium azide
2.5 NF	= 2.5 µg per ml 2-nitrofluorene
2.5 AAN	= 2.5 µg per ml 2-aminoanthracene
25 AAC	= 25 µg per ml 9-aminoacridine

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- E.C. WP2uvrA pkm 101
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 10PD
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	106	98	83	****	95.7	11.7	1.00
2	0.390000	98	98	110	****	102.0	6.9	1.07
3	0.781000	87	91	92	****	90.0	2.6	0.94
4	1.56200	96	89	103	****	96.0	7.0	1.00
5	3.12500	94	98	119	****	103.7	13.4	1.08
6	6.25000	119	103	98	****	106.7	11.0	1.11
7	12.5000	105	102	108	****	105.0	3.0	1.10
8	25.0000	86	90	109	****	95.0	12.3	0.99
9	50.0000	110	108	85	****	101.0	13.9	1.06
10	+CONTROL	254	264	288	****	268.7	17.5	2.81

Comments
========

BACKGROUND OK.

SBGR.92.257

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- E.C. WP2uvrA pkm 101
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5BP
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107

+S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	130	113	112	****	118.3	10.1	1.00
2	3.90600	127	99	110	****	112.0	14.1	0.95
3	7.81200	123	124	127	****	124.7	2.1	1.05
4	15.6250	121	125	121	****	122.3	2.3	1.03
5	31.2500	115	116	111	****	114.0	2.6	0.96
6	62.5000	111	104	149	****	121.3	24.2	1.03
7	125.000	114	97	108	****	106.3	8.6	0.90
8	250.000	96	84	91	****	90.3	6.0	0.76
9	500.000	6	8	4	****	6.0	2.0	0.05
10	+CONTROL	463	396	414	****	424.3	34.7	3.59

Comments
========

BACKGROUND REDUCED AT 500uG/ML.

Experiment number 5765

Run date 12-NOV-92
Start date 10-NOV-92

Bacterial strain :- E.C. WP2uvrA pkm 101
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 10 PD
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	76	87	73	****	78.7	7.4	1.00
2	0.781000	76	95	72	****	81.0	12.3	1.03
3	1.56200	93	86	76	****	85.0	8.5	1.08
4	3.12500	88	79	93	****	86.7	7.1	1.10
5	6.25000	79	97	77	****	84.3	11.0	1.07
6	12.5000	93	83	73	****	83.0	10.0	1.06
7	25.0000	82	90	84	****	85.3	4.2	1.08
8	50.0000	49	57	54	****	53.3	4.0	0.68
9	100.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	358	570	438	****	455.3	107.1	5.79

Comments
========

BACKGROUND OK UP TO 50 uG/ML;100 BACKGROUND ONLY.

Experiment number 5765

Run date 12-NOV-92
Start date 10-NOV-92

Bacterial strain :- E.C. WP2uvrA pkm 101
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5BP
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107

+S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	95	93	93	****	93.7	1.2	1.00
2	3.90600	66	71	105	****	80.7	21.2	0.86
3	7.81200	88	96	101	****	95.0	6.6	1.01
4	15.6250	79	70	89	****	79.3	9.5	0.85
5	31.2500	97	84	105	****	95.3	10.6	1.02
6	62.5000	75	97	110	****	94.0	17.7	1.00
7	125.000	79	83	68	****	76.7	7.8	0.82
8	250.000	72	54	73	****	66.3	10.7	0.71
9	500.000	8	4	5	****	5.7	2.1	0.06
10	+CONTROL	624	701	679	****	668.0	39.7	7.13

Comments
========

BACKGROUND OK UP 250 uG/ML:500 B.REDUCED.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 1535
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 1 NaN3
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	13	14	13	****	13.3	0.6	1.00
2	0.390000	11	10	14	****	11.7	2.1	0.88
3	0.781000	14	15	18	****	15.7	2.1	1.18
4	1.56200	14	11	19	****	14.7	4.0	1.10
5	3.12500	13	11	11	****	11.7	1.2	0.88
6	6.25000	18	12	19	****	16.3	3.8	1.23
7	12.5000	17	8	11	****	12.0	4.6	0.90
8	25.0000	17	15	19	****	17.0	2.0	1.27
9	50.0000	13	13	25	****	17.0	6.9	1.27
10	+CONTROL	328	441	519	****	429.3	96.0	32.20

Comments
========

BACKGROUND OK.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 1535
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5 AAN
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107

+S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	12	13	11	****	12.0	1.0	1.00
2	3.90600	12	12	8	****	10.7	2.3	0.89
3	7.81200	11	15	10	****	12.0	2.6	1.00
4	15.6250	17	11	13	****	13.7	3.1	1.14
5	31.2500	15	8	7	****	10.0	4.4	0.83
6	62.5000	6	13	9	****	9.3	3.5	0.78
7	125.000	10	11	11	****	10.7	0.6	0.89
8	250.000	0	0	0	****	0.0	0.0	0.00
9	500.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	122	118	116	****	118.7	3.1	9.89

Comments
========

BACKGROUND OK UP TO 31.25 uG/ML;62.5 AND 125 B.REDUCED;250 REDUCED B.ONLY;500 NO GROWTH.

Experiment number 5765

Run date 09-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 1535
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 1 NaN3
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	10	16	9	****	11.7	3.8	1.00
2	0.781000	13	7	11	****	10.3	3.1	0.89
3	1.56200	10	10	17	****	12.3	4.0	1.06
4	3.12500	7	11	9	****	9.0	2.0	0.77
5	6.25000	15	11	11	****	12.3	2.3	1.06
6	12.5000	12	12	8	****	10.7	2.3	0.91
7	25.0000	8	15	9	****	10.7	3.8	0.91
8	50.0000	2	3	0	****	1.7	1.5	0.14
9	100.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	670	647	618	****	645.0	26.1	55.29

Comments
========

BACKGROUND OK UP TO 25 uG/ML;50 B.REDUCED;100 NO GROWTH.

Experiment number 5765

Run date 09-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 1535
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5 AAN
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107

+S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	11	10	10	****	10.3	0.6	1.00
2	0.976000	9	6	11	****	8.7	2.5	0.84
3	1.95300	10	8	9	****	9.0	1.0	0.87
4	3.90600	8	6	6	****	6.7	1.2	0.65
5	7.81200	9	11	9	****	9.7	1.2	0.94
6	15.6250	5	6	10	****	7.0	2.6	0.68
7	31.2500	14	8	7	****	9.7	3.8	0.94
8	62.5000	6	8	5	****	6.3	1.5	0.61
9	125.000	6	5	14	****	8.3	4.9	0.81
10	+CONTROL	145	172	145	****	154.0	15.6	14.90

Comments
========

BACKGROUND OK UP TO 62.5 uG/ML;125 B.REDUCED.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 1537
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 25 AAC
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	9	10	10	****	9.7	0.6	1.00
2	0.390000	8	7	9	****	8.0	1.0	0.83
3	0.781000	9	7	12	****	9.3	2.5	0.97
4	1.56200	10	9	7	****	8.7	1.5	0.90
5	3.12500	7	8	9	****	8.0	1.0	0.83
6	6.25000	6	6	8	****	6.7	1.2	0.69
7	12.5000	6	7	4	****	5.7	1.5	0.59
8	25.0000	8	6	4	****	6.0	2.0	0.62
9	50.0000	4	3	1	****	2.7	1.5	0.28
10	+CONTROL	412	726	322	****	486.7	212.1	50.34

Comments
========

BACKGROUND OK UP TO 25 uG/ML;50 B.REDUCED.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 1537
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 10 NR
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107

+S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	9	11	11	****	10.3	1.2	1.00
2	3.90600	9	7	5	****	7.0	2.0	0.68
3	7.81200	9	9	7	****	8.3	1.2	0.81
4	15.6250	10	4	9	****	7.7	3.2	0.74
5	31.2500	8	6	8	****	7.3	1.2	0.71
6	62.5000	6	4	7	****	5.7	1.5	0.55
7	125.000	5	3	6	****	4.7	1.5	0.45
8	250.000	0	0	0	****	0.0	0.0	0.00
9	500.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	525	519	461	****	501.7	35.3	48.55

Comments
========

BACKGROUND OK UP TO 62.5 uG/PLATE;125 B.REDUCED;250 AND 500
NO GROWTH.

Experiment number 5765

Run date 09-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 1537
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 25 AAC
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	13	8	9	****	10.0	2.6	1.00
2	0.781000	5	9	6	****	6.7	2.1	0.67
3	1.56200	5	11	6	****	7.3	3.2	0.73
4	3.12500	6	10	5	****	7.0	2.6	0.70
5	6.25000	9	8	5	****	7.3	2.1	0.73
6	12.5000	9	5	8	****	7.3	2.1	0.73
7	25.0000	12	7	7	****	8.7	2.9	0.87
8	50.0000	0	0	0	****	0.0	0.0	0.00
9	100.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	112	92	112	****	105.3	11.5	10.53

Comments
========

BACKGROUND OK UP TO 25 uG/ML;50 B.REDUCED ONLY;100
NO GROWTH.

Experiment number 5765

Run date 09-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 1537
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 10 NR
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107 +S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	9	10	8	****	9.0	1.0	1.00
2	0.976000	8	12	6	****	8.7	3.1	0.96
3	1.95300	8	4	6	****	6.0	2.0	0.67
4	3.90600	8	9	7	****	8.0	1.0	0.89
5	7.81200	9	6	12	****	9.0	3.0	1.00
6	15.6250	6	7	5	****	6.0	1.0	0.67
7	31.2500	8	6	7	****	7.0	1.0	0.78
8	62.5000	7	7	4	****	6.0	1.7	0.67
9	125.000	2	2	4	****	2.7	1.2	0.30
10	+CONTROL	217	171	88	****	158.7	65.4	17.63

Comments
========

BACKGROUND OK UP TO 62.5 uG/ML;125 B.REDUCED.

Experiment number 5765

Run date 09-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 1538
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5 NF
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	10	11	14	****	11.7	2.1	1.00
2	0.781000	11	10	10	****	10.3	0.6	0.89
3	1.62500	12	12	18	****	14.0	3.5	1.20
4	3.12500	10	9	12	****	10.3	1.5	0.89
5	6.25000	13	14	14	****	13.7	0.6	1.17
6	12.5000	12	10	12	****	11.3	1.2	0.97
7	25.0000	8	10	10	****	9.3	1.2	0.80
8	50.0000	10	8	7	****	8.3	1.5	0.71
9	100.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	386	418	441	****	415.0	27.6	35.57

Comments
========

BACKGROUND OK UP TO 25 uG/ML;50 B.REDUCED;100
NO GROWTH.

Experiment number 5765

Run date 09-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 1538
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5 BP
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107

+S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	17	15	12	****	14.7	2.5	1.00
2	0.976000	17	14	21	****	17.3	3.5	1.18
3	1.95300	13	18	14	****	15.0	2.6	1.02
4	3.90600	12	10	18	****	13.3	4.2	0.91
5	7.81200	19	15	10	****	14.7	4.5	1.00
6	15.6250	16	16	23	****	18.3	4.0	1.25
7	31.2500	15	11	20	****	15.3	4.5	1.05
8	62.5000	11	7	12	****	10.0	2.6	0.68
9	125.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	271	293	269	****	277.7	13.3	18.93

Comments
========

BACKGROUND OK UP TO 31.25 uG/ML;62.5 B.REDUCED;125 NO GROWTH.

Experiment number 5765

Run date 09-NOV-92
Start date 05-NOV-92

Bacterial strain :- TA 1538
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5 NF
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	12	21	15	****	16.0	4.6	1.00
2	0.781000	14	10	7	****	10.3	3.5	0.65
3	1.56200	12	11	17	****	13.3	3.2	0.83
4	3.12500	14	8	15	****	12.3	3.8	0.77
5	6.25000	21	14	12	****	15.7	4.7	0.98
6	12.5000	16	14	13	****	14.3	1.5	0.90
7	25.0000	14	14	12	****	13.3	1.2	0.83
8	50.0000	8	5	6	****	6.3	1.5	0.40
9	100.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	325	339	417	****	360.3	49.6	22.52

Comments
========

BACKGROUND OK UP TO 25 uG/ML;50 B.REDUCED;100 NO GROWTH.

Experiment number 5765

Run date 09-NOV-92
Start date 05-NOV-92

Bacterial strain :- TA 1538
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5 BP
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107 +S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	16	15	15	****	15.3	0.6	1.00
2	0.976000	10	17	18	****	15.0	4.4	0.98
3	1.95300	15	11	11	****	12.3	2.3	0.80
4	3.90600	16	13	20	****	16.3	3.5	1.07
5	7.81200	11	16	22	****	16.3	5.5	1.07
6	15.6250	24	13	21	****	19.3	5.7	1.26
7	31.2500	22	13	15	****	16.7	4.7	1.09
8	62.5000	5	12	8	****	8.3	3.5	0.54
9	125.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	238	268	252	****	252.7	15.0	16.48

Comments
========

BACKGROUND OK UP TO 31.25 uG/ML;62.5 B.REDUCED;125 NO GROWTH.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 98
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5NF
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	15	5	18	****	12.7	6.8	1.00
2	0.390000	15	18	12	****	15.0	3.0	1.18
3	0.781000	15	11	12	****	12.7	2.1	1.00
4	1.56200	21	18	9	****	16.0	6.2	1.26
5	3.12500	17	14	21	****	17.3	3.5	1.37
6	6.25000	15	19	12	****	15.3	3.5	1.21
7	12.5000	14	13	11	****	12.7	1.5	1.00
8	25.0000	10	13	13	****	12.0	1.7	0.95
9	50.0000	2	1	3	****	2.0	1.0	0.16
10	+CONTROL	251	306	256	****	271.0	30.4	21.39

Comments
========

BACKGROUND OK UP TO 25uG/ML :50uG/ML BACKGROUND REDUCED.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 98
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5BP
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107 +S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	19	20	17	****	18.7	1.5	1.00
2	3.90600	17	18	21	****	18.7	2.1	1.00
3	7.81200	0	20	12	****	10.7	10.1	0.57
4	15.6250	19	17	17	****	17.7	1.2	0.95
5	31.2500	16	19	20	****	18.3	2.1	0.98
6	62.5000	11	9	8	****	9.3	1.5	0.50
7	125.000	10	7	7	****	8.0	1.7	0.43
8	250.000	14	10	15	****	13.0	2.6	0.70
9	500.000	11	8	15	****	11.3	3.5	0.61
10	+CONTROL	245	265	237	****	249.0	14.4	13.34

Comments
========

BACKGROUND REDUCED AT DOSES OF 31.25uG/ML AND ABOVE.

Experiment number 5765

Run date 06-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 98
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5NF
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	18	14	19	****	17.0	2.6	1.00
2	0.781000	12	16	11	****	13.0	2.6	0.76
3	1.56200	20	13	20	****	17.7	4.0	1.04
4	3.12500	11	19	14	****	14.7	4.0	0.86
5	6.25000	16	13	11	****	13.3	2.5	0.78
6	12.5000	14	15	14	****	14.3	0.6	0.84
7	25.0000	12	14	9	****	11.7	2.5	0.69
8	50.0000	5	8	9	****	7.3	2.1	0.43
9	100.000	0	0	0	****	0.0	0.0	0.00
10	+CONTROL	265	381	407	****	351.0	75.6	20.65

Comments
========

BACKGROUND OK. UP TO 25uG/ML,50uG/ML BACKGROUND REDUCED,
100ug/ml NO GROWTH.

Experiment number 5765

Run date 06-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 98
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5BP
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107 +S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	18	12	8	****	12.7	5.0	1.00
2	0.976000	24	10	15	****	16.3	7.1	1.29
3	1.95300	21	10	17	****	16.0	5.6	1.26
4	3.90600	15	15	26	****	18.7	6.4	1.47
5	7.81200	13	18	16	****	15.7	2.5	1.24
6	15.6250	16	10	12	****	12.7	3.1	1.00
7	31.2500	12	12	15	****	13.0	1.7	1.03
8	62.5000	12	19	21	****	17.3	4.7	1.37
9	125.000	17	23	5	****	15.0	9.2	1.18
10	+CONTROL	252	246	300	****	266.0	29.6	21.00

Comments
========

BACKGROUND REDUCED AT 31.25uG/ML AND ABOVE.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 100
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5NaN3
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	109	93	106	****	102.7	8.5	1.00
2	0.390000	126	98	121	****	115.0	14.9	1.12
3	0.781000	109	101	96	****	102.0	6.6	0.99
4	1.56200	106	94	109	****	103.0	7.9	1.00
5	3.12500	113	99	95	****	102.3	9.5	1.00
6	6.25000	104	120	108	****	110.7	8.3	1.08
7	12.5000	106	126	114	****	115.3	10.1	1.12
8	25.0000	111	80	93	****	94.7	15.6	0.92
9	50.0000	76	96	66	****	79.3	15.3	0.77
10	+CONTROL	630	632	567	****	609.7	37.0	5.94

Comments
========

BACKGROUND REDUCED AT 50uG/ML.

Experiment number 5765

Run date 02-NOV-92
Start date 30-OCT-92

Bacterial strain :- TA 100
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5BP
Solvent control :- DMSO
Source of S9 :- RAT.BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107 +S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	117	158	91	****	122.0	33.8	1.00
2	3.90600	123	106	121	****	116.7	9.3	0.96
3	7.81200	110	147	109	****	122.0	21.7	1.00
4	15.6250	95	119	125	****	113.0	15.9	0.93
5	31.2500	125	123	106	****	118.0	10.4	0.97
6	62.5000	109	116	155	****	126.7	24.8	1.04
7	125.000	117	110	94	****	107.0	11.8	0.88
8	250.000	6	7	6	****	6.3	0.6	0.05
9	500.000	2	2	3	****	2.3	0.6	0.02
10	+CONTROL	401	408	412	****	407.0	5.6	3.34

Comments
========

BACKGROUND REDUCED AT 500uG/ML.

Experiment number 5765

Run date 06-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 100
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 2.5NaN3
Solvent control :- DMSO

Data capture :- AUTOMATIC

SOP No. :- 107 -S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	98	76	90	****	88.0	11.1	1.00
2	0.781000	78	92	91	****	87.0	7.8	0.99
3	1.56200	97	93	111	****	100.3	9.5	1.14
4	3.12500	77	83	98	****	86.0	10.8	0.98
5	6.25000	125	88	115	****	109.3	19.1	1.24
6	12.5000	103	116	106	****	108.3	6.8	1.23
7	25.0000	80	88	105	****	91.0	12.8	1.03
8	50.0000	91	97	70	****	86.0	14.2	0.98
9	100.000	5	0	0	****	1.7	2.9	0.02
10	+CONTROL	316	408	286	****	336.7	63.6	3.83

Comments
========

BACKGROUND OK. UP TO 50uG/ML,100uG/ML BACKGROUND REDUCED.

Experiment number 5765

Run date 06-NOV-92
Start date 04-NOV-92

Bacterial strain :- TA 100
Test compound :- MONOBUTYL-P-CRESOL
Positive control :- 5BP
Solvent control :- DMSO
Source of S9 :- RAT,BATCH 38
Inducer :- AROCLOR
Data capture :- AUTOMATIC

SOP No. :- 107 +S9

Dose No.	Dose	Count 1	Count 2	Count 3	Count 4	Mean	S.D.	TM/CM
1	0	104	113	104	****	107.0	5.2	1.00
2	3.90600	105	85	108	****	99.3	12.5	0.93
3	7.81200	118	141	105	****	121.3	18.2	1.13
4	15.6250	114	99	91	****	101.3	11.7	0.95
5	31.2500	111	149	126	****	128.7	19.1	1.20
6	62.5000	156	117	159	****	144.0	23.4	1.35
7	125.000	154	117	168	****	146.3	26.4	1.37
8	250.000	127	103	118	****	116.0	12.1	1.08
9	500.000	5	11	6	****	7.3	3.2	0.07
10	+CONTROL	282	350	374	****	335.3	47.7	3.13

Comments
========

BACKGROUND OK. UP TO 250uG/ML,500uG/ML BACKGROUND REDUCED.

APPENDIX 2

COMPOUND CONTROL AND FORMULATION CHEMISTRY REPORT

Title of main report:	Monobutyl-p-cresol : Bacterial mutagenicity studies.
Experiment number:	5765
Responsible Practitioner:	D[redacted]s
Participants:	[redacted]
Summary:	Data concerning the test and control substances and their formulations are reported.

1. Test substance

1.1 Identity of the test substance

NAME	Monobutyl-p-cresol 2-tert.Butyl-4-methylphenol
CODE NUMBER	[redacted]
C.A.S. NUMBER	2409-55-4
BATCH (& OTHER) NUMBERS	1 90; Date of sampling 21/7/92 Indent No. 9200/9006
TOXICOLOGY REF. NUMBER	ST92/303
SOURCE	Derfesa, Derivados Fenolicos SA, Spain
DATE RECEIVED	2nd September 1992
APPEARANCE	Low melting point solid
CHARACTERIZATION	Actual analysis 2-tert.Butyl-p-cresol 99.38 % 6-tert.Butyl-m-cresol 0.39 % 2,6-Di-tert.butyl-p-cresol 0.23 % Water (Karl-Fischer 0.012 % Ref. Certificate ex Derfesa dated 28/7/92 No claim of GLP compliance is made in respect of these data.
DATE RELEASED	17th September 1992

1.2 Storage of this test substance

Following its arrival in Compound Control this test substance was stored in the dark at room temperature. Following receipt of the test substance data sheet the storage temperature was changed to between 0 and 5°C. This was effective from 18th September 1992.

1.3 Stability of this test substance

The test substance data sheet supplied for the sample gave a shelf life of one year when stored in the dark at 0-5°C. On this basis I consider that it was stable for the duration of this study.

2. Control substances

Details of the control substances released for use in this study are shown below.

Name	Code No.	CAS No.	Batch No.	ST No.	Source
3,4-Benzopyrene	B1,008-0	[50-32-8]	KV01511KV	ST89/255	Aldrich Chemical Co. Ltd.
Sodium Azide	10369	[26628-22-8]	3637840J	ST88/237	BDH Ltd.
Neutral Red	34056 4A	[553-24-2]	2143532L	ST91/310	BDH Ltd.
Potassium Dichromate	10202	[7778-50-9]	4272700J	ST88/236	BDH Ltd.
2-Nitrofluorene	N1,675-4	[607-57-8]	1666	ST88/067	Aldrich Chemical Co. Ltd.
9-Aminoacridine hydrochloride	A3,840-1	[52417-22-8]	05311PP	ST88/065	Aldrich Chemical Co. Ltd.
9-Aminoacridine hydrochloride	A3,840-1	[52417-22-8]	02205AV	ST90/371	Aldrich Chemical Co. Ltd.
2-Aminoanthracene	A3,880-0	[613-13-8]	1514TD	ST88/066	Aldrich Chemical Co. Ltd.

3. Formulation of the test and control substances

Data concerning formulations of the test and control substances are given below.

Test or Control Substance	Vehicle +	Concentration (mg/ml)	Shelf Life	Basis of Shelf Life Estimate
Monobutyl-p-cresol	DMSO	300 - 0.011	1 day	Assessed
3,4-Benzopyrene	DMSO	0.5	4 weeks	High performance liquid chromatography *
Sodium Azide	Water	0.1	4 weeks	Ultra-violet/visible spectrophotometry *
Neutral Red	Water	1	4 weeks	Ultra-violet/visible spectrophotometry *
Potassium Dichromate	Water	1	4 weeks	Assessed
2-Nitrofluorene	DMSO	0.25	4 weeks	High performance liquid chromatography *
9-Aminoacridine hydrochloride	DMSO	2.5	4 weeks	Mutagenic activity
2-Aminoanthracene	DMSO	0.25	4 weeks	Mutagenic activity

+ DMSO, dimethyl sulphoxide, was "SpS" grade supplied by Romil Ltd.

* Stability studies carried out at Sittingbourne Research Centre

4. Stability of formulations of the test and control substances

The test substance is a substituted phenol. Chemical interaction with DMSO is not likely to be significant at room temperature in the few hours that would elapse between the preparation of the formulations and their use.

Stability studies which provided the data on which the shelf lives of the control substances are based were not carried out on the batches used in this study, but are considered to be independent of the batch.

On the basis of the above I consider that the formulations of the test and control substances used in this study were stable for their period of use.

[redacted], BA, PhD, CChem, MRSC
Compound Controller / Formulation Chemist

Date : 17 November 1992

SBGR.92.257

Monobutyl-p-cresol: Bacterial mutagenicity studies

DISTRIBUTION

SIPC (ODLC/731)	3
SICC (CMSE/22)	5
SICC (CBDR)	1
SICC (CMSE/321)	1
SICC (CMKSF/12)	1
SICM (CMFS/234)	5
Shell Dev. Co. (SDWR)	9
CRC (RSOK)	1
SIPM (HSE/51)	2
KSLA (IDC/122)	2
SIPM (HSE/225)	1

[redacted] SBGR.92.257

FURTHER DETAILS FOR DATA BASE ENTRY

INDEX TERMS:
15. TOXICOLOGY
10. CHEMICALS
16. RESEARCH & DEVELOPMENT

KEYWORDS: Monobutyl-p-cresol, Mutagenicity, Salmonella typhimurium, Escherichia coli,

Shell Research
Sittingbourne

GROUP RESEARCH REPORT

SBGR.95.081

500 70 649 SP4904

Butyl-p-cresol: physicochemical properties

Fisk PR

SCEL, CMKSF

GROUP RESEARCH REPORT

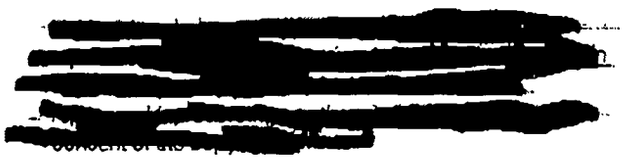
SBGR.95.081

500-70 649 SP4904

Butyl-p-cresol: physicochemical properties

Fisk PR

SCEL, CMKSF



[redacted] [redacted]

DOCUMENT TYPE:	GROUP RESEARCH REPORT	
DOCUMENT NUMBER:	SBGR.95.081	
TITLE:	Butyl-p-cresol: physicochemical properties	
AUTHOR(S):	Fisk PR	SE/1
REVIEWED BY:	Lyne RL	SE/1
PARTICIPANT(S):	-	-
PROJECT NUMBER:	SP4904	
SUB PROJECT:	5974	
PROJECT TITLE:	Fine Chemicals - Ecotox	
SPONSOR:	SCEL, CMKSF	
BUDGET CODE:	500 70 649	
SOURCE:	Shell Research Limited, Sittingbourne Research Centre.	
ORIGINATING DEPT:	Safety & Environmental Research Department	
DATE:	June 1995	

HS/682

Butyl-p-cresol: physicochemical properties

(Study number 5974)

SUMMARY:

A number of physicochemical properties of butyl-p-cresol have been determined. The results obtained were as follows.

Water solubility : 0.325 g/l at 20°C, obtained by the flask method.

Octanol-water partition coefficient, P_{ow} : log P_{ow} = 3.97, estimated by the LOGKOW program.

The study was carried out at Huntingdon Research Centre.

C.J. Kroese, Manager & Director Research,
Shell Research Limited,
Sittingbourne Research Centre,
Sittingbourne, Kent, ME9 8AG, England

Date : 20/6/95

TEXT:

INTRODUCTION

The determination of several physicochemical properties of butyl-p-cresol has been requested, and the work carried out is reported herein. The study was performed at Huntingdon Research Centre, monitored by the Environmental Research Department.

[redacted]

SBGR.95.081

[redacted]

SLL 296/942293

BUTYL-P-CRESOL

PHYSICO-CHEMICAL PROPERTIES

Sponsor

Shell Research Ltd.,
Sittingbourne Research Centre,
Sittingbourne,
Kent,
ME9 8AG,
ENGLAND.

Testing facility

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued 4 May 1995

Sponsor's representative

Dr. P. Fisk

CONTENTS

[redacted]

SBGR.95.081

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

J. B. Betteley

John M.T. Betteley,
Study Director,
Huntingdon Research Centre Ltd.

4 May 1995
Date

QUALITY ASSURANCE STATEMENT

Certain studies such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Date(s) of inspection	22 - 29 September 1993 12 - 18 May 1994
Date(s) of reporting inspection findings to the Study Director and HRC Management	29 September 1993 27 May 1994
Date of reporting audit findings to the Study Director and HRC Management	12 September 1994

D. J. Dams

David J. Dams,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

2-May-1995
Date

[redacted]
SBGR.95.081

SLL 296/942293

RESPONSIBLE PERSONNEL

We the undersigned hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

David A. Howes, B.Sc., Ph.D.,
Section Head, Physicochemical Testing,
Department of Environmental Analysis. D A Howes

John M.T. Betteley,
Higher National Certificate,
Study Director,
Department of Environmental Analysis. J. Betteley

Stephen J. Young, L.R.S.C.,
Study Supervisor,
Department of Environmental Analysis. S. Young

SUMMARY

A study was performed to determine some physico-chemical properties of butyl-p-cresol. The methods followed are described in the EEC Methods for determination of physico-chemical properties, Directive 92/69/EEC (OJ No. L383A, 29.12.92), Part A, Methods A1 - A17. The physico-chemical properties which have been determined in this study are detailed below, together with the result obtained for each test.

EEC Method	Test	Result
A6	Water solubility	0.325 g/l at 20°C
A8	Partition coefficient	Log P = 3.97 (calculated)

INTRODUCTION

This study was designed to determine some physico-chemical properties of butyl-p-cresol. Information on physico-chemical properties is important in the assessment of the potential effects of a substance both in the work place and in the environment.

The study was conducted in compliance with EEC Methods for the determination of physico-chemical properties Directive 92/69/EEC (OJ No. L383A, 29.12.92), Part A, Methods A1 - A17. The physico-chemical properties investigated were: test A6 water solubility and test A8 partition coefficient (calculated).

The protocol was approved by the Study Director and HRC Management on 25 January 1994 and by the Sponsor on 7 February 1994.

The experimental phase of the study was undertaken between 21 February and 7 March 1994.

[redacted]
SBGR.95.081

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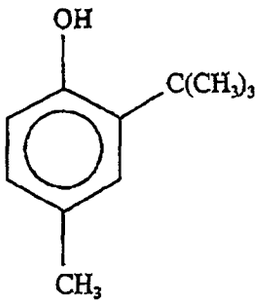
TEST SUBSTANCE

Identity:	-Butyl-p-cresol
Chemical name:	2-tert-Butyl-p-cresol
Batch number:	1 - 90
Sponsor's code number:	ST 92/303
Expiry:	3 February 1995
Purity:	99.38%
Appearance:	Off white crystalline solid
Storage conditions:	In the dark at 4°C
Date received:	17 February 1994

Structure:



[redacted]

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WATER SOLUBILITY (A6)

EXPERIMENTAL PROCEDURE

METHOD

Water solubility was determined by the flask-stirring method.

DEFINITION AND UNITS

The solubility in water is specified by the saturation mass concentration of the substance in water, and is a function of temperature. Solubility is specified in units of mass per volume of solution. The SI unit is kg/m^3, g/l may also be used.

INSTRUMENTATION AND APPARATUS

HPLC system:	Pump, Model 305 with 805 manometric module, Gibson Medical Electronics
	Autosampler, WISP Model 712, Waters Associates
	Detector, Model 115, Gibson Medical Electronics
	Data handling system, Model 1020 Perkin Elmer Nelson
	Printer, Diconix 180 si, Kodak
Analytical balance:	Model R160P, Sartorius Instruments
Magnetic stirrers:	Model AS607 controller with AS623 stirrer plate, Stem Corporation
pH meter:	Model 245, Corning
Water baths:	Stainless steel, manufactured by the Department of Bio-Medical Engineering, HRC
Water bath heaters:	Type TM, Grant Instruments

General laboratory glassware.

REAGENTS

Methanol:	Fisons HPLC grade.
Calibration buffers: (pH 7 and 9)	Fisons plc.
Water:	Glass-distilled at HRC.

PERFORMANCE OF THE TEST

Test substance (*ca* 0.175 g) was combined with distilled water (100 ml) in each of six conical flasks. The flasks were firmly stoppered and stirred at 30°C prior to equilibration at the test temperature of 20°C. Distilled water was also set stirring alongside tests 1 and 3 to act as blanks. The flask contents were stirred as indicated below:

Test	Pre-equilibration (30°C)	Equilibration (20°C)
1A, 1B, Blank 1	3 days	1 day
2A, 2B	2 days	1 day
3A, 3B, Blank 2	1 day	1 day

ANALYSIS

The contents of each flask was filtered using GF/F filter paper. An aliquot (10 ml) of each clear solution was pipetted into a 100 ml volmetric flask and made to volume with methanol/water 50/50 v/v.

An aliquot (10 ml) of each of the blank solutions was similarly diluted.

The pH of the remaining filtrates was measured.

The diluted solutions were analysed by HPLC using the conditions detailed overleaf.

HPLC CONDITIONS

Column:	C18, 5 μ particle size, 15 cm $\times$ 4.6 mm id.
Mobile phase:	Methanol/water (75/25, v/v).
Flow rate:	1.0 ml/min.
Pressure:	1230 psi.
Analytical wavelength:	225 nm.
Injection volume:	20 μl.

Under these conditions the test substance chromatographed as a single peak with an approximate retention volume of 4.8 ml.

RECOVERY SOLUTIONS

A stock recovery solution of concentration 1001.1 μg/ml was prepared by weighing test substance 0.10011 g in a 100 ml volumetric flask and dissolving in, and making to volume with methanol/water 50/50 v/v.

An aliquot (3.0 ml) was diluted to 100 ml in a volumetric flask methanol/water 50/50 v/v. This procedure was performed in duplicate.

PREPARATION OF CALIBRATION SOLUTIONS

A stock calibration solution of concentration 1000.8 μg/ml was prepared by weighing test substance (0.10008 g) into a 100 ml volumetric flask and dissolving in, and making to volume with methanol/water 50/50 v/v.

Calibration solutions in the range 40.032 to 5.0040 μg/ml were prepared by dilution of the stock solution with methanol/water 50/50 v/v.

CALCULATION

The peak response of test substance in each calibration solution chromatogram was measured and a calibration curve constructed by linear regression of standard response versus standard concentration (μg/ml). The response of the peak observed at the characteristic retention volume for the test substance in the sample chromatograms was measured and the water solubility (g/l) calculated using the equation below:

Regression equation: $Y = I + Sx$

Tests

$$\text{Water solubility (g/l)} = \frac{Y - I}{S} \times F \times 10^{-3}$$

Recovery solutions

$$\text{Recovery \%} = \frac{Y - I}{S} \times \frac{F}{C} \times 100$$

where
- Y = integrated peak area of sample chromatogram
- I = intercept derived from linear regression of calibration data
- S = slope derived from linear regression of calibration data
- x = concentration of calibration standard (μg/ml)
- F = dilution factor (10) for tests; $\frac{100}{3}$ for recovery solution
- 10^{-3} = conversion factor (μg/ml to g/l)
- C = concentration of stock recovery solution (1001.1 μg/ml)

ARCHIVES

All raw data and other documents generated at HRC during the course of this work, together with a copy of this Final Report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England.

RESULTS

Calibration data are given in Table 1 providing a slope of 372800 an intercept of 15650 and a correlation coefficient of 0.9999. There was no trend in the residuals and therefore the detector response is linear over the standard concentration range. Analytical data are given in Table 2 yielding water solubility results of 0.312 to 0.346 g/l (mean 0.325 g/l) and a pH range of 7.62 - 7.71. No time dependence of test solutions was observed.

Recovery data are given in Table 3 showing recovery values of 101.4% and 101.5%. The analytical data have not been corrected for the mean value obtained.

Typical chromatograms are shown in Figures 1 and 2.

No peak was observed in either of the blank solutions.

CONCLUSION

The water solubility of butyl-p-cresol has been determined as:

0.325 g/l at 20°C (mean pH 7.65)

TABLE 1

Calibration data

Test substance concentration (µg/ml)	Integrated peak area					
40.032	15088562					15019040
30.024	11145512	11098664	11113738	11130518	11144024	11195885
20.016	7555368					7518256
10.008	3809254					3783400
5.0040	1857640					1868744
Correlation coefficient	0.9999					
Slope	372800					
Intercept	15650					

TABLE 2

Analytical data

Test sample	Integrated peak area	Water solubility (g/l)	Mean water solubility for each test (g/l)	Mean water solubility for tests 1, 2 and 3 (g/l)	pH
1A	12844372	0.344	0.328		7.71
1B	11642902	0.312			7.65
2A	11639768	0.312	0.329	0.325	7.62
2B	12906386	0.346			7.62
3A	12024112	0.322	0.317		7.68
3B	11632718	0.312			7.62

TABLE 3

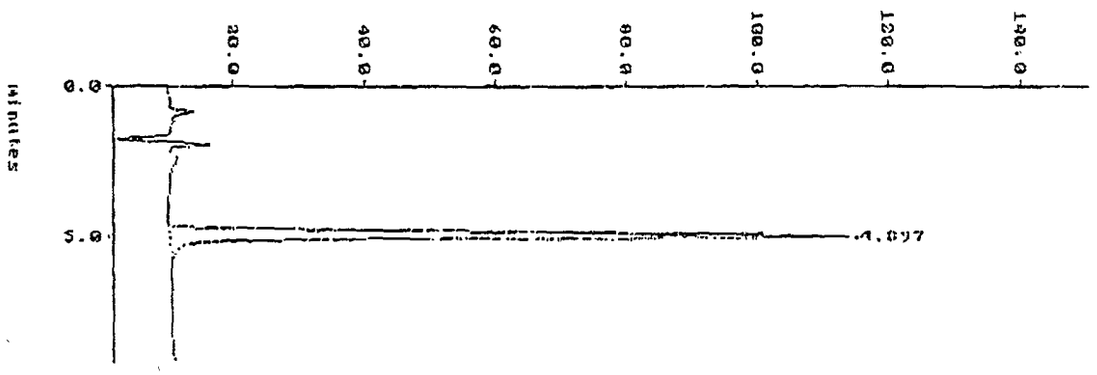
Analytical recovery data

Test solution	Integrated peak area	Analysed concentration (µg/ml)	Nominal concentration (µg/ml)	% Recovery
1	11381168	1016.3	1001.1	101.5
2	11364310	1014.8	1001.1	101.4

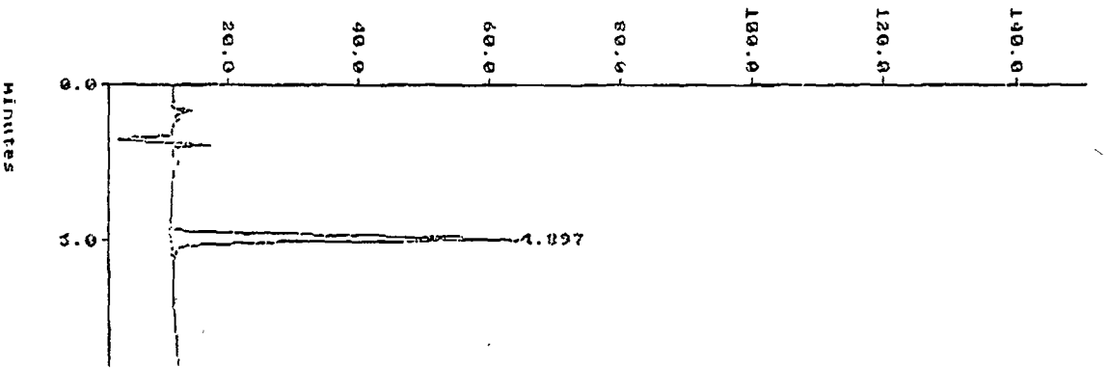
FIGURE 1

Typical chromatograms: calibration solutions

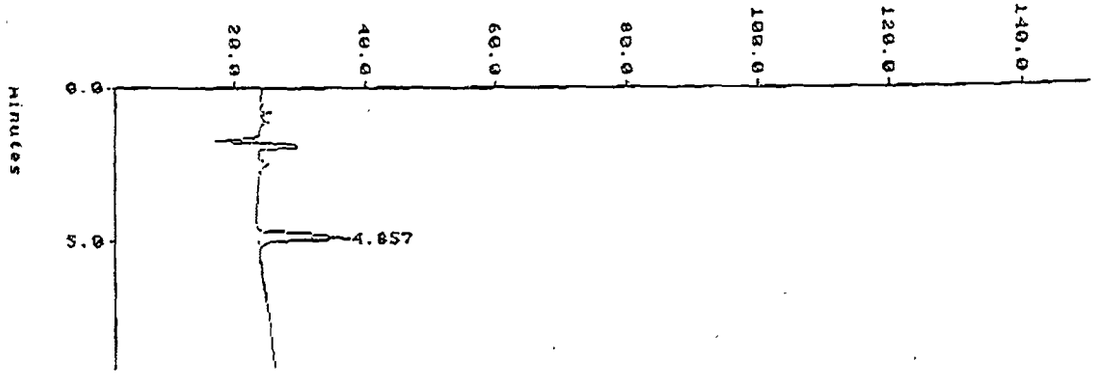
40.032 $\mu g/ml^{-1}$ Standard



20.016 $\mu g/ml^{-1}$ Standard



5.0040 $\mu g/ml^{-1}$ Standard



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SBGR.95.081

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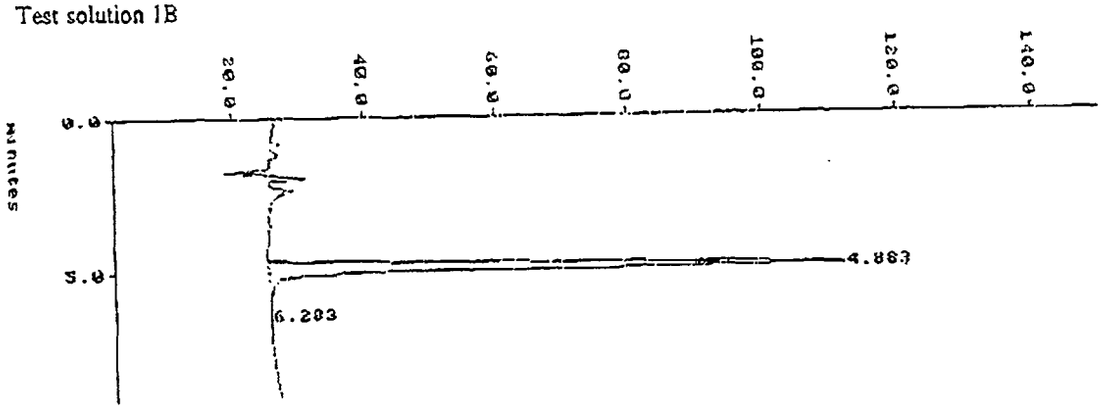
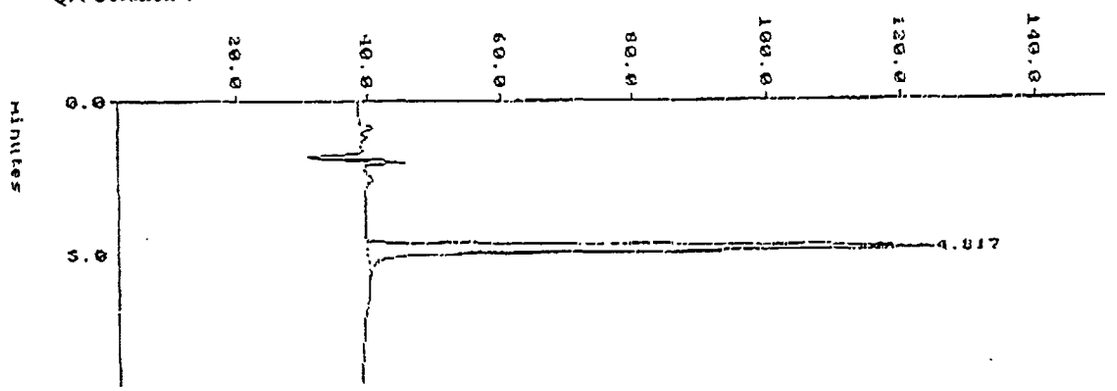
FIGURE 2

Typical chromatograms: test solutions

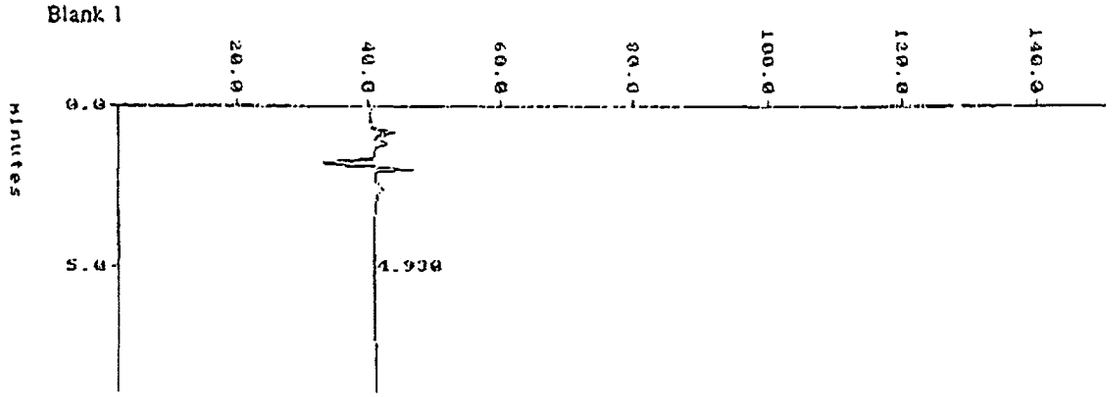
Test solution 1B



QA Solution 1



Blank 1



PARTITION COEFFICIENT (A8)

EXPERIMENTAL PROCEDURE

METHOD

The partition coefficient was determined by a calculation method using computer software from Syracuse Research Corporation, U.S.A.

DEFINITIONS AND UNITS

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two phase system consisting of two largely immiscible solvents - in this case, n-octanol and water.

$$P = \frac{\text{Concentration in octanol}}{\text{Concentration in water}}$$

The partition coefficient is the quotient of two concentrations and is usually given in the form of its logarithm to base ten (log P).

INSTRUMENTATION

Computer:	Model F20 IBM with model 6312002 monitor, IBM.
Printer:	Model XB-2420, Star.
Software:	LOGKOW program, Syracuse Research Corporation, USA.

PERFORMANCE OF THE TEST

The structure of butyl-p-cresol was entered into the computer program in the form of SMILES (simplified molecular input line entry system) notation and the estimate of the partition coefficient calculated by summation of all relevant group contributions.

As a means of checking the program's validity two further compounds (para-cresol and tertiary butyl benzene) were also entered. The estimated values for these two molecules (both exhibiting structural similarities to butyl-p-cresol) were compared to experimentally derived literature values.

ARCHIVES

All raw data and other documents generated at HRC during the course of this work, together with a copy of this final report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England.

RESULTS

The LOG KOW program gave a calculated partition coefficient for butyl-p-cresol of Log P = 3.97. The output of the program is shown in Figure 3.

The validation compounds gave the following results;

	LOGKOW program result	Experimentally determined literature value*
Para cresol	2.0601	1.93
Tertiary butyl benzene	3.9025	4.11

* Leo, Hansch, Elkins. *Chemical Reviews*, 1971, vol.71, No.6

The closeness of these results indicates that the program is estimating the group contributions correctly.

CONCLUSION

Butyl-p-cresol has a partition coefficient of Log P = 3.97.

FIGURE 3

Computer printout of partition coefficient

SMILES : c1c(C)cc(C(C)(C)(C))c(O)c1
CHEM : Butyl P-Cresol
MOL FOR: C11 H16 O1
MOL WT : 164.25

	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	4	-CH3 [aliphatic carbon]	0.5473	2.1892
Frag	6	Aromatic Carbon	0.2940	1.7640
Frag	1	-OH [hydroxy, aromatic attach]	-0.4802	-0.4802
Frag	1	-tert Carbon [3 or more carbon attach]	0.2676	0.2676
Const		Equation Constant		0.2290
			Log Kow =	3.9696

[redacted]

SBGR.95.081

FURTHER DETAILS FOR DATA BASE ENTRY

INDEX TERMS:
10. Chemicals
15. Toxicology
16. Research and Development

KEYWORDS: Water solubility, Partition coefficient

[redacted]

SBGR.95.081

Butyl-p-cresol: physicochemical properties

DISTRIBUTION

[redacted]

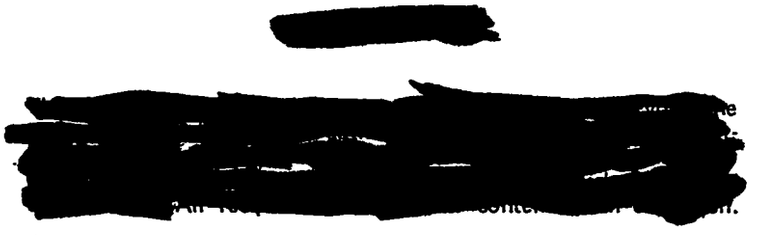
1
3
1
5
9
2
2

GROUP RESEARCH REPORT

TLGR.80.156

TOXICITY STUDIES WITH MINING CHEMICALS: IN VITRO GENOTOXICITY STUDIES WITH SODIUM ISOPROPYL XANTHATE

SICC/CIMS Budget Ref: 50070695



SHELL RESEARCH LIMITED, LONDON

SITTINGBOURNE RESEARCH CENTRE

SHELL TOXICOLOGY LABORATORY (TUNSTALL)

[redacted]

[redacted]

Shell Toxicology Laboratory (Tunstall)

Group Research Report TLGR.80.156 Experiment Number 1MX-1628

Title: Toxicity studies with Mining Chemicals: In vitro genotoxicity studies with sodium isopropyl xanthate.

Introduction: This report describes the results of a series of in vitro tests to investigate the genotoxicity of sodium isopropyl xanthate. The assays include [redacted] agar overlay bacterial tests, a liquid culture assay for mitotic gene conversion in yeast and a cytogenetic study in cultured rat liver cells.

Date study started: 23rd July, 1979

Study Director: [redacted]

Authors: [redacted]

Responsible Practitioners: [redacted] Microbiologist; Technician (Cytogenetics); Formulation Chemist; Technician (Formulation); Compound Controller

Reviewer: [redacted]

Summary: The mutagenic activity of sodium isopropyl xanthate was investigated in agar layer cultures of Salmonella typhimurium and Escherichia coli bacterial tester strains and in liquid cultures of the yeast, Saccharomyces cerevisiae. Assays were performed both in the presence and absence of S9 microsomal fraction obtained from a liver homogenate from rats pre-treated with Aroclor. Monolayer slide cultures of rat liver (RL4) cells were cultured for 24 hours in culture medium containing sodium isopropyl xanthate; metaphase cells were analysed for structural chromosome aberrations.

The results indicate that sodium isopropyl xanthate did not induce mutation in bacteria, gene conversion in yeast or

chromosome damage in rat liver cells under the conditions of the assays described.

Microscope slide preparations of RL_4 cells are stored in the Chemical Mutagenesis Slide Archive, the raw data from all studies and the final report are stored in the Record, Shell Toxicology Laboratory (Tunstall).

[redacted]

[redacted], B.V.M.S., M.R.C.V.S., D.V.M., Ph.D., F.R.C. Path., F.I. Biol.
Director, Shell Toxicology Laboratory (Tunstall)
Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG.

Date: May 1981

[redacted]

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PROCEDURES

The microorganisms and the procedures are described in STL SOP 28/01/001 and STL SOP 28/01/004. The microorganisms used were Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100, Escherichia coli WP_2 and WP_2 uvr A and Saccharomyces cerevisiae JD1.

The rat liver (RL_4) cell culture and procedures are described in SOP 28/01/003.

Methods

a) Bacterial mutation study

20 µl volumes of 0.01, 0.1, 1.0, 10 or 100 mg/ml solutions of sodium isopropyl xanthate in distilled water were added to top agar mix to give final amounts of 0.2, 2.0, 20, 200 or 2000 µg per plate in both the presence and absence of rat liver S9 fraction. The cultures were incubated at 37°C for 48 hours before the revertant colonies were counted.

An additional experiment was carried out to study the influence of sodium isopropyl xanthate on the activity of the monooxygenase system in the rat liver S9 fraction. The positive control compound benzo(a)pyrene was incorporated in a conventional agar assay with Aroclor-induced rat liver S9 fraction and either 200 or 2000 µg per plate of sodium isopropyl xanthate using S. typhimurium TA 98. The amounts of benzo(a)pyrene tested were 5, 10, 20 µg per plate. After incubation, the revertant colonies were counted and the influence of sodium isopropyl xanthate on the benzo(a)pyrene-induced reversion frequency was determined.

b) Saccharomyces gene conversion assay

Liquid suspension cultures were dosed with 20 µl (without S9 mix) or 25 µl (with S9 mix) of 1, 10, 50, 100 or 250 mg/ml solutions of sodium isopropyl xanthate in water to give final concentrations of 0.01, 0.1, 0.5, 1.0 or 2.5 mg/ml both with and without the incorporation of rat liver S9 fraction. After 1 h incubation without S9 fraction and after 1 h and 4 h incubation with S9 fraction, the cultures were seeded onto the appropriate culture media for the selection of revertant colonies. After 3 days incubation at 30°C the numbers of revertant colonies were counted.

c) Rat liver chromosome assay

RL_4 slide cultures were exposed to culture medium containing sodium isopropyl xanthate at final concentrations of 0.25, 0.5, 1.0, 2.0 or 4.0 µg/ml. After 24 hours the cultures were processed for chromosome analysis and, where possible, 100 cells analysed from each of three cultures per dose group.

Materials

Sodium isopropyl xanthate was obtained from Shell Santiago, Chile (Batch No. Secado 1734) and prepared for use as solutions in sterile distilled water.

Benzo(a)pyrene, Batch No. KL 62991, was obtained from Koch-Light Laboratories and prepared as 0.25, 0.5 and 1.0 mg/ml solutions in dimethyl sulphoxide (DMSO).

Cyclophosphamide, Batch No. 74841, was obtained from Koch-Light Laboratories and prepared as a 25 mg/ml solution in sterile distilled water.

Neutral red was obtained from G.T. Gurr Ltd., London and prepared as as 1 mg/ml solution in water.

4-Nitroquinoline-N-oxide, Batch No. 3757-10, was a gift from Dr. J. Ashby, ICI Ltd., CTL, Alderley Edge, Cheshire and prepared as 0.01, 0.1 and 1 mg/ml solutions in DMSO.

Sodium azide, Batch No. 40, was supplied by Fisons Laboratory Equipment, Loughborough, Leics., and prepared as a 1 mg/ml solution in distilled water.

7,12-Dimethylbenzanthracene, Batch No. A6B, was supplied by Eastman-Kodak Co., Kirby, Liverpool, and prepared for use as a 0.5 mg/ml solution in DMSO.

DISCUSSION

Solutions of sodium isopropyl xanthate in water were shown to be stable for at least 4 hours (Appendix 3), which was the maximum period between preparation of the formulations and their incorporation in the assay systems.

In the bacterial assays, sodium isopropyl xanthate did not induce reverse gene mutations in the Salmonella or Escherichia tester strains.

It was considered that under certain in vitro experimental conditions, sodium isopropyl xanthate may inhibit mono-oxygenase enzyme activity (D. Hutson, personal communication). In order to ascertain whether sodium isopropyl xanthate interfered with the activity of rat liver S9 microsomal enzymes in the microbial assays, the test compound was studied in a mutation experiment using benzo(a)pyrene. Amounts of 200 or 2000 µg per plate of sodium isopropyl xanthate were incorporated in the agar overlay together with standard S9 mix, Salmonella typhimurium TA 98 and benzo(a)pyrene. A reduction in the mutagenic activity of benzo(a)pyrene was observed on the addition of 2000 µg per plate sodium isopropyl xanthate but not with 200 µg per plate. The activity of the S9 fraction was therefore not affected by the inclusion of sodium isopropyl xanthate at amounts up to 200 µg per plate.

Studies with sodium isopropyl xanthate in Saccharomyces cerevisiae JD1 showed that the compound did not induce mitotic gene conversion.

Sodium isopropyl xanthate did not induce detectable chromosome damage in the rat liver chromosome assay.

CONCLUSION

Applications of sodium isopropyl xanthate at amounts up to 2000 µg per plate did not increase the reverse mutation rate of Escherichia coli WP_2 and WP_2 uvr A or Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100 in vitro in the presence or absence of a rat liver microsomal activation system.

Exposure of Saccharomyces cerevisiae JD1 to sodium isopropyl xanthate in vitro in liquid culture at concentrations up to 2.5 mg/ml did not result in any consistent increase in the rate of mitotic gene conversion either in the presence or absence of a rat liver microsomal activation system.

As there was no increase in the frequency of chromatid gaps, chromatid breaks or total chromatid aberrations in cultures exposed to sodium isopropyl xanthate it is concluded that the compound did not induce chromosome damage in cultured rat liver (RL_4) cells.

The results show that sodium isopropyl xanthate does not induce reverse gene mutation in bacteria, mitotic gene conversion in yeast or chromosome damage in cultured rat liver cells under the experimental conditions described.

[redacted] M.I. Biol.
Study Director
Date: 1/5/81.

[redacted] Walker
Responsible Practitioner
Date: 5.5.81

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APPENDIX 1

MICROBIOLOGY REPORT

Title: [redacted] Toxicity studies with Mining Chemicals: In vitro microbial mutation studies with sodium isopropyl xanthate.

Responsible Practitioner: [redacted] T. M. Brooks

Work done:

The mutagenic activity of sodium isopropyl xanthate was investigated in agar layer cultures of Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100, Escherichia coli WP_2 and WP_2 uvr A and in liquid cultures of Saccharomyces cerevisiae JD1 both with and without the incorporation of a rat liver microsomal activation system.

The influence of sodium isopropyl xanthate was also studied on the mutation frequency of benzo(a)pyrene using Salmonella typhimurium TA 98 in the presence of rat liver S9 fraction.

Results

a) Bacterial mutation study (Tables 1.1a, 1.1b, 1.1c and 1.1d)

The addition of sodium isopropyl xanthate to agar layer cultures of Escherichia coli WP_2 and WP_2 uvr A and Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100 both with and without the incorporation of a rat liver microsomal fraction (S9) did not lead to an increase in the reverse mutation frequency in any of the strains. The amounts of sodium isopropyl xanthate tested were 0.2, 2.0, 20, 200 or 2000 µg per plate.

The activity of the S9 mix and of the strains TA 98, TA 100 and TA 1538 was monitored by treating cultures with a known positive control compound benzo(a)pyrene which requires metabolic activation before it is able to induce gene mutation. The sensitivity of TA 1537 was monitored by the indirect mutagen neutral red and the E. coli strains and TA 1535 were monitored by testing with the direct-acting mutagens 4-nitroquinoline-N-oxide and sodium azide respectively.

The addition of 2000 µg per plate sodium isopropyl xanthate to 5, 10 or 20 µg per plate benzo(a)pyrene in the presence of rat liver S9 fraction resulted in an inhibition in response of strain TA 98 to benzo(a)pyrene-mediated mutagenicity (Table 1.1d). This effect was not seen with the addition of 200 µg per plate sodium isopropyl xanthate.

b) Saccharomyces gene conversion assay (Tables 1.2a and 1.2b)

The addition of sodium isopropyl xanthate to liquid suspension cultures of Saccharomyces cerevisiae JD1 with or without the addition of a rat liver microsomal fraction did not induce a consistent increase in mitotic gene conversion. The concentrations of sodium isopropyl xanthate tested were 0.01, 0.1, 0.5, 1.0 and 2.5 mg/ml. Treatment with 4-nitroquinoline-N-oxide, a direct-acting mutagen, and cyclophosphamide, and indirect mutagen, was shown to induce mitotic gene conversion.

[redacted], M.I. Biol.
Responsible Practitioner
Date: 1/5/81

Table 1.1a – Relative reverse mutation rates in Escherichia coli WP_2 and WP_2 uvrA and Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100 after treatment with sodium isopropyl xanthate in the plate incorporated assay

Micro-organisms	Experiment Number	Sodium isopropyl xanthate µg per plate																		
		Without Microsomal Activiation (-S9)									With Microsomal Activation (+S9)									
		0.2	2.0	20	200	2000	NaN_3(a) 20 µg	BP(b) 20 µg	NQO(c) 20 µg	NR(d) 20 µg	0.2	2.0	20	200	2000	NaN_3(a) 20 µg	BP(b) 20 µg	NQO(c) 20 µg	NR(d) 20 µg
E. coli WP_2	5	0.5	1.0	0.8	1.1	0.8	–	–	11.8*	–	1.0	1.0	1.0	1.4	0.7	–	–	1.5	–
	8	1.1	0.7	0.9	1.3	1.1	–	–	33.3*	–	1.0	1.0	1.0	1.1	0.7	–	–	1.5	–
E. coli WP_2 uvr A	1	1.0	0.8	1.0	1.2	0.9	–	–	6.8*	–	0.8	0.9	0.9	1.5	0.9	–	–	36.2*	–
	5	1.1	1.0	1.0	1.3	1.2	–	–	18.8*	–	1.0	1.2	1.0	1.1	0.6	–	–	27.9*	–
S. typhimurium TA 1535	2	1.0	0.9	1.1	1.0	0	73.6*	–	–	–	0.9	0.9	0.8	0.9	0.7	105.3*	–	–	–
	4	1.0	1.5	1.3	1.7	0.5	179.6*	–	–	–	0.7	0.9	1.3	1.5	1.0	78.1*	–	–	–
S. typhimurium TA 1537	8	0.9	1.3	0.9	1.2	0.4	–	–	–	2.3	0.9	0.9	0.6	1.0	0.9	–	–	–	19.0*
	9	1.4	0.8	1.6	1.5	0.3	–	–	–	1.8	1.0	1.4	1.2	1.1	1.0	–	–	–	25.4*
S. typhimurium TA 1538	2	1.2	0.8	0.9	0.8	0.3	–	1.3	–	–	0.7	0.8	0.9	0.8	0.2	–	3.2*	–	–
	3	0.8	0.7	1.2	1.3	0.4	–	0.8	–	–	1.4	1.3	1.2	0.9	0.1	–	4.2*	–	–
S. typhimurium TA 98	6	0.6	1.3	0.8	1.0	0.9	–	1.0	–	–	1.0	1.0	0.9	0.9	0.6	–	3.3*	–	–
	7	1.5	1.4	1.1	1.7	1.3	–	1.4	–	–	0.8	0.8	1.0	1.5	0.7	–	3.1*	–	–
S. typhimurium TA 100	4	0.8	0.8	1.0	1.1	0.4	–	1.2	–	–	1.1	1.0	1.1	1.2	0.4	–	2.7*	–	–
	5	1.1	1.1	1.0	0.8	0.7	–	0.7	–	–	1.2	0.9	1.1	1.1	0.3	–	3.0*		

Results are expressed as a ratio: $\frac{\text{Mean number of revertant colonies per treated plate}}{\text{Mean number of revertant colonies per control plate}}$

(a) Sodium azide
(b) Benzo(a)pyrene
(c) 4-Nitroquinoline-N-oxide
(d) Neutral red

* Reproducible values of 2.5 x control value or greater are considered to indicate a mutagenic response.

– Not tested

Table 1.1b - Mean number of revertants per plate after treatment of bacteria with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO), benzo(a)pyrene (BP), sodium azide (NaN_3) or neutral red (NR) in the plate incorporated assay

µg/plate	*Escherichia coli* WP_2			
	Experiment 5		Experiment 8	
	-S9	+S9	-S9	+S9
0	10.0 ± 5.1	11.3 ± 4.0	5.8 ± 2.2	6.5 ± 2.4
0.2	5.3 ± 3.1	10.8 ± 2.6	6.3 ± 2.5	6.3 ± 2.1
2	9.8 ± 5.0	11.3 ± 2.6	3.8 ± 2.2	6.3 ± 0.5
20	7.5 ± 4.4	11.5 ± 1.7	5.0 ± 1.4	6.3 ± 1.5
200	10.5 ± 1.9	15.8 ± 4.5	7.5 ± 2.9	7.3 ± 1.3
2000	7.8 ± 1.7	8.0 ± 6.8	6.3 ± 2.8	4.5 ± 2.6
20 NQO	117.8 ± 48.4	16.8 ± 3.5	193.0 ± 91.0	9.5 ± 1.7

µg/plate	*Escherichia coli* WP_2 *uvr A*			
	Experiment 1		Experiment 5	
	-S9	+S9	-S9	+S9
0	11.5 ± 3.3	19.3 ± 9.3	11.0 ± 1.6	17.8 ± 2.2
0.2	11.0 ± 4.2	15.8 ± 7.2	11.8 ± 2.2	18.3 ± 2.5
2	9.0 ± 5.4	17.3 ± 8.0	11.0 ± 4.4	22.0 ± 3.7
20	11.3 ± 2.2	16.5 ± 11.8	10.5 ± 4.4	17.0 ± 2.9
200	13.5 ± 5.2	29.3 ± 7.0	13.8 ± 0.5	19.8 ± 4.3
2000	10.8 ± 4.8	18.3 ± 5.3	13.3 ± 3.8	10.0 ± 3.9
20 NQO	78.5 ± 75.8	699.0 ± 146.9	207.3 ± 201.0	496.0 ± 117.4

µg/plate	*Salmonella typhimurium* TA 100			
	Experiment 4		Experiment 5	
	-S9	+S9	-S9	+S9
0	62.0 ± 16.5	65.8 ± 12.4	71.3 ± 12.3	74.3 ± 8.7
0.2	48.5 ± 9.8	75.0 ± 19.8	77.5 ± 19.9	85.8 ± 24.0
2	52.0 ± 1.8	63.0 ± 15.7	77.0 ± 19.9	69.0 ± 7.4
20	61.8 ± 5.7	74.0 ± 7.9	70.8 ± 6.8	85.3 ± 18.0
200	69.3 ± 4.3	79.3 ± 10.9	54.8 ± 10.0	80.0 ± 23.8
2000	22.5 ± 8.6	25.8 ± 12 1	49.5 ± 8.7	21.3 ± 13.6
20 BP	73.3 ± 11.3	179.3 ± 22.3	51.5 ± 9.1	222.3 ± 81.2

Table 1.1b Contd

µg/plate	Salmonella typhimurium TA 1535			
	Experiment 2		Experiment 4	
	-S9	+S9	-S9	+S9
0	10.8 ± 4.0	10.8 ± 2.8	5.5 ± 1.3	13.0 ± 2.4
0.2	10.8 ± 5.0	9.5 ± 2.6	5.5 ± 1.7	9.3 ± 1.9
2	9.3 ± 3.0	9.3 ± 6.3	8.3 ± 3.7	12.0 ± 2.9
20	12.3 ± 4.8	8.8 ± 6.8	7.3 ± 3.8	16.8 ± 6.4
200	10.8 ± 3.0	9.5 ± 7.0	9.5 ± 3.7	19.5 ± 8.6
2000	0.3 ± 0.5	7.5 ± 4.9	3.0 ± 0.8	12.8 ± 3.2
20 NaN_3	794.8 ± 81.6	1137.5 ± 128.1	988.0 ± 88.3	1015.0 ± 67.6
µg/plate	Salmonella typhimurium TA 1538			
	Experiment 2		Experiment 3	
	-S9	+S9	-S9	+S9
0	8.3 ± 1.7	41.3 ± 9.2	2.0 ± 1.6	12.3 ± 5.6
0.2	10.3 ± 1.9	30.8 ± 4.1	1.5 ± 2.4	17.0 ± 2.7
2	6.8 ± 2.6	34.8 ± 5.0	1.3 ± 0.5	16.0 ± 7.0
20	7.3 ± 3.0	37.0 ± 7.7	2.3 ± 1.5	15.0 ± 5.2
200	7.0 ± 1.8	32.0 ± 3.7	2.5 ± 1.3	10.5 ± 3.0
2000	2.5 ± 1.9	7.8 ± 2.2	0.8 ± 0.5	1.8 ± 1.5
20 BP	10.8 ± 3.8	131.5 ± 16.4	1.5 ± 0.6	51.3 ± 10.2
µg/plate	Salmonella typhimurium TA 98			
	Experiment 6		Experiment 7	
	-S9	+S9	-S9	+S9
0	5.3 ± 1.7	10.3 ± 4.3	8.3 ± 2.4	14.8 ± 4.3
0.2	3.3 ± 2.6	10.0 ± 1.4	12.3 ± 5.7	12.3 ± 3.2
2	7.0 ± 4.8	10.0 ± 2.7	11.8 ± 4.3	11.8 ± 1.0
20	4.5 ± 3.9	9.0 ± 2.9	9.3 ± 2.5	14.3 ± 4.8
200	5.5 ± 1.7	9.3 ± 2.2	14.0 ± 5.9	22.5 ± 6.1
2000	4.8 ± 4.3	5.8 ± 3.5	11.0 ± 4.1	10.3 ± 2.6
20 BP	5.5 ± 5.4	34.3 ± 11.8	11.3 ± 2.2	45.5 ± 6.4

Table 1.1b Contd

µg/plate	Salmonella typhimurium TA 1537			
	Experiment 8		Experiment 9	
	-S9	+S9	-S9	+S9
0	5.5 ± 2.1	7.8 ± 3.5	4.3 ± 2.2	6.5 ± 1.3
0.2	4.8 ± 2.5	6.8 ± 2.2	6.0 ± 2.4	6.8 ± 1.9
2	7.3 ± 2.6	7.3 ± 2.2	3.3 ± 1.9	9.0 ± 0.8
20	4.8 ± 1.5	4.3 ± 2.1	6.8 ± 1.7	7.8 ± 3.3
200	6.8 ± 3.0	7.8 ± 1.0	6.3 ± 1.7	7.3 ± 3.5
2000	2.3 ± 1.5	7.0 ± 2.4	1.3 ± 1.3	6.3 ± 2.9
20 NR	12.5 ± 6.6	148.3 ± 24.8	7.8 ± 1.0	165.0 ± 48.7

Table 1.1c - Number of revertants per plate after treatment of bacteria with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO), benzo(a)pyrene (BP), sodium azide (NaN_3) or neutral red (NR) in the plate incorporated assay - raw data

µg/plate	*Escherichia coli* WP_2															
	Experiment 5								Experiment 8							
	-S9				+S9				-S9				+S9			
0	5	10	8	17	8	9	11	17	7	8	3	5	8	9	5	4
0.2	5	8	7	1	8	9	13	13	6	3	7	9	9	6	4	6
2	3	15	10	11	10	9	15	11	5	3	1	6	6	6	7	6
20	2	11	11	6	11	14	11	10	5	4	4	7	5	8	5	7
200	13	11	9	9	9	18	18	18	7	4	8	11	7	7	9	6
2000	8	10	6	7	17	9	1	5	8	3	9	5	2	8	3	5
20 NQO	105	184	68	114	13	15	21	18	202	143	110	317	10	10	7	11

µg/plate	*Escherichia coli* WP_2 *uvr A*															
	Experiment 1								Experiment 5							
	-S9				+S9				-S9				+S9			
0	16	11	11	8	E	30	13	15	9	11	11	13	17	17	21	16
0.2	15	14	6	9	22	22	9	10	11	15	11	10	18	19	15	21
2	13	3	6	14	26	22	11	10	6	9	16	13	26	22	17	23
20	12	12	8	13	34	13	11	8	7	9	9	17	17	21	14	16
200	20	8	15	11	31	29	37	20	14	13	14	14	17	26	19	17
2000	5	13	9	16	25	18	12	18	17	10	10	16	5	14	12	9
20 NQO	36	192	45	41	883	581	580	752	51	100	180	498	587	568	329	500

µg/plate	*Salmonella typhimurium* TA 100															
	Experiment 4								Experiment 5							
	-S9				+S9				-S9				+S9			
0	50	50	85	63	76	67	72	48	72	71	86	56	66	84	79	68
0.2	57	57	40	40	53	64	88	95	98	91	63	58	110	102	60	71
2	50	53	51	54	60	74	42	76	53	69	89	97	74	60	66	76
20	65	68	56	58	69	66	83	78	81	67	67	68	75	74	112	80
200	72	70	72	63	90	66	75	86	42	52	60	65	115	66	75	64
2000	17	34	15	24	13	38	34	18	61	42	52	44	5	38	23	19
20 BP	71	62	89	71	181	156	171	209	42	63	47	54	316	233	118	222

Table 1.1c Contd

µg/plate	Salmonella typhimurium TA 1535															
	Experiment 2								Experiment 4							
	-S9				+S9				-S9				+S9			
0	13	15	6	9	12	9	8	14	6	5	7	4	15	10	15	12
0.2	12	11	16	4	12	9	6	11	3	7	6	6	8	12	8	9
2	6	13	10	8	3	18	8	8	13	8	8	4	12	13	8	15
20	19	8	12	10	17	11	6	1	4	11	4	10	15	15	11	26
200	15	8	10	10	9	13	16	0	7	14	6	11	12	31	14	21
2000	1	0	0	0	13	10	5	2	3	2	4	3	10	15	10	10
20 NaN_3	799	888	803	689	986	1090	1191	1283	1081	1016	870	985	951	1013	987	1109

µg/plate	Salmonella typhimurium TA 1538															
	Experiment 2								Experiment 3							
	-S9				+S9				-S9				+S9			
0	8	9	6	10	31	49	36	49	2	4	0	2	10	12	7	20
0.2	10	9	13	9	32	28	27	36	0	1	5	0	18	19	18	13
2	3	9	8	7	34	38	28	39	1	2	1	1	7	17	16	24
20	4	8	11	6	44	42	35	27	1	3	1	4	20	19	11	10
200	5	6	8	9	34	36	28	30	2	3	1	4	9	9	9	15
2000	5	3	1	1	10	5	9	7	0	1	1	1	4	1	1	1
20 BP	10	6	15	12	108	137	146	135	1	2	2	1	51	57	37	60

µg/plate	Salmonella typhimurium TA 98															
	Experiment 6								Experiment 7							
	-S9				+S9				-S9				+S9			
0	5	7	3	6	13	13	4	11	8	5	10	10	11	18	19	11
0.2	6	1	1	5	10	9	12	9	7	19	15	8	11	17	11	10
2	14	6	5	3	9	14	8	9	8	8	16	15	12	13	11	11
20	1	3	4	10	5	10	12	9	6	9	10	12	9	20	16	12
200	4	7	7	4	12	10	7	8	20	14	16	6	17	25	30	18
2000	2	2	4	11	2	4	7	10	13	5	12	14	10	14	9	8
20 BP	13	6	1	2	44	43	31	19	9	10	12	14	55	42	42	43

Table 1.1c Contd

µg/plate	Salmonella typhimurium TA 1537															
	Experiment 8								Experiment 9							
	-S9				+S9				-S9				+S9			
0	5	3	6	8	9	4	12	6	2	5	3	7	5	7	8	6
0.2	8	4	2	5	10	5	6	6	3	9	6	6	7	8	4	8
2	6	11	7	5	5	8	10	6	2	2	3	6	9	8	10	9
20	3	4	6	6	4	2	4	7	9	6	5	7	7	4	8	12
200	6	6	4	11	7	8	7	9	6	8	7	4	11	9	6	3
2000	4	3	1	1	5	5	10	8	1	3	1	0	10	6	6	3
20 NR	8	12	22	8	168	169	118	138	7	8	7	9	128	119	216	197

E = plate lost due to experimental error.

Table 1.1d - The influence of sodium isopropyl xanthate (SIX) on mixed function oxidase-mediated mutagenicity of benzo(a)pyrene using S. typhimurium TA 98

Benzo(a)pyrene µg/plate	Mean number of revertants per plate		Number of revertants per plate (raw data)	
	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 2000 µg per plate SIX	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 2000 µg per plate SIX
Experiment 1				
0	29.3 ± 4.7	13.3 ± 3.2	28 25 28 36	12 12 11 18
5	130.8 ± 13.0	90.0 ± 25.2	142 114 127 140	65 101 74 120
10	192.3 ± 30.4	108.0 ± 45.3	205 147 213 204	138 85 153 56
20	139.0 ± 31.4	32.8 ± 10.5	135 183 129 109	23 38 25 45
Experiment 2				
0	15.3 ± 2.9	10.0 ± 1.4	17 17 11 16	12 10 9 9
5	72.0 ± 3.9	44.8 ± 11.6	70 77 73 68	30 42 57 50
10	58.0 ± 22.1	30.0 ± 12.8	31 49 74 78	19 44 42 47
20	52.0 ± 14.7	21.3 ± 12.5	34 46 64 64	11 30 10 34
	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 200 µg per plate Six	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 200 µg per plate Six
Experiment 3				
0	25.3 ± 5.7	23.3 ± 5.4	21 21 33	30 20 18
5	102.0 ± 24.2	119.8 ± 24.3	128 79 84	108 92 146
10	212.8 ± 18.5	284.8 ± 18.0	222 201 234	290 270 308
20	333.8 ± 50.8	263.5 ± 55.9	266 354 329	281 209 230

Table 1.2a - Mitotic gene conversion in liquid cultures of Saccharomyces cerevisiae JD1 after treatment with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO) or cyclophosphamide (CP) in the presence and absence of rat liver S9 fraction

mg compound per ml	Survivors x 10^4 (per plate)	HISTIDINE LOCUS			TRYPTOPHAN LOCUS		
		Revertants per plate	Revertants per 10^6 survivors	Ratio(1) over control	Revertants per plate	Revertants per 10^6 survivors	Ratio(1) over control
Experiment 1A 1 hr -S9 at room temperature							
0	206	3.3	1.6	-	17.5	8.5	-
0.01	117	1.3	1.1	1	6.5	5.6	1
0.1	177	4.0	2.3	1	19.7	11.1	1
0.5	182	1.5	0.8	1	10.5	5.8	1
1.0	168	1.3	0.8	1	15.8	9.4	1
2.5	176	0	0	-	6.3	3.6	1
0.001 NQO	5	60.8	1216.0	760*	259.3	5186.0	610*
Experiment 1B 1 hr +S9 at 37°C							
0	145	2.5	1.7	-	7.0	4.8	-
0.01	123	1.5	1.2	1	5.8	4.7	1
0.1	129	1.3	1.0	1	5.3	4.1	1
0.5	130	1.3	1.0	1	4.0	3.1	1
1.0	187	0.3	0.2	1	4.8	2.6	1
2.5	103	0	0	-	0.5	0.5	1
10 CP	119	2.5	2.1	1	11.3	9.5	2
Experiment 1C 4 hr +S9 at 37°C							
0	115	2.8	2.4	-	19.5	17.0	-
0.01	159	1.3	0.8	1	7.7	4.8	1
0.1	127	0.8	0.6	1	5.0	3.9	1
0.5	120	2.5	2.1	1	14.0	11.7	1
1.0	92	0.5	0.5	1	7.8	8.5	1
2.5	59	0	0	-	0	0	-
10 CP	106	22.0	20.8	9*	189.0	178.3	10*
Experiment 2A 1 hr -S9 at room temperature							
0	154	6.8	4.4	-	41.3	26.8	-
0.01	148	5.8	3.9	1	54.8	37.0	1
0.1	147	8.3	5.6	1	47.0	32.0	1
0.5	102	2.3	2.3	1	41.8	41.0	2
1.0	130	0	0	-	35.8	27.5	1
2.5	82	0	0	-	0	0	-
0.0001 NQO	101	15.8	15.6	4*	97.8	96.8	4*
Experiment 2B 1 hr +S9 at 37°C							
0	112	4.0	3.6	-	48.5	43.3	-
0.01	106	4.8	4.5	1	39.5	37.3	1
0.1	153	5.3	3.5	1	39.5	25.8	1
0.5	142	3.3	2.3	1	37.5	26.4	1
1.0	118	0.8	0.7	1	30.0	25.4	1
2.5	123	0	0	-	0.5	0.4	1
10 CP	134	8.0	6.0	2	43.3	32.3	1
Experiment 2C 4 hr +S9 at 37°C							
0	130	4.0	3.1	-	42.3	32.5	-
0.01	165	4.3	2.6	1	40.8	24.7	1
0.1	147	4.8	3.3	1	38.3	26.1	1
0.5	124	6.5	5.2	2	39.3	31.7	1
1.0	133	2.5	1.9	1	29.5	22.2	1
2.5	125	0	0	-	0.8	0.6	1
10 CP	70	23.8	34.0	11*	223.0	318.6	10*

$$\text{Ratio}^{(1)} = \frac{\text{Mean number of revertants per } 10^6 \text{ survivors per treated plate}}{\text{Mean number of revertants per } 10^6 \text{ survivors per control plate}}$$

* Reproducible values of greater than twice the control value are considered to indicate a mutagenic response.

Table 1.2b - Mitotic gene conversion in liquid cultures of Saccharomyces cerevisiae JD1 after treatment with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO) or cyclophosphamide (CP) in the presence and absence of rat liver S9 fraction - raw data

mg/ml	Revertants per plate								Survivors x 10^4 (per plate)		
	HISTIDINE LOCUS				TRYPTOPHAN LOCUS						
Expt 1A 1 hr -S9 at room temperature											
0	5	0	5	3	24	11	C	C	239	214	165
0.01	1	1	1	2	9	5	3	9	149	85	E
0.1	3	8	4	1	17	34	8	C	156	173	202
0.5	1	1	1	3	11	10	8	13	210	153	E
1.0	2	2	1	0	8	18	15	22	154	185	164
5.0	0	0	0	0	0	14	7	4	186	209	133
0.001 NQO	48	65	78	52	310	286	172	269	6	8	1
Expt 1B 1 hr +S9 at 37°C											
0	1	1	3	5	8	8	4	8	161	143	132
0.01	3	1	2	0	8	4	4	7	96	145	127
0.1	1	3	1	0	7	2	5	7	124	112	152
0.5	0	1	1	3	4	2	2	8	170	85	135
1.0	0	0	0	1	7	5	5	2	141	208	212
5.0	0	0	0	0	2	0	0	0	103	82	125
10 CP	1	4	3	2	6	13	11	15	150	100	107
Expt 1C 4 hr +S9 at 37°C											
0	1	3	4	3	24	34	13	7	158	106	80
0.01	1	1	2	1	7	C	6	10	161	133	184
0.1	1	1	0	1	4	2	9	5	126	125	130
0.5	4	2	3	1	17	16	12	11	161	121	77
1.0	0	0	0	2	6	8	6	11	58	108	110
5.0	0	0	0	0	0	0	0	0	31	82	65
10 CP	21	C	23	C	197	224	162	173	131	67	121

Table 1.2b - Contd

Expt 2A -S9 for 1 hr at room temperature											
0	3	8	5	11	49	39	34	43	154	138	169
0.01	5	7	4	7	54	72	42	51	187	135	122
0.1	7	9	7	10	46	44	48	50	133	165	144
0.5	4	2	2	1	38	53	37	39	130	107	68
1.0	0	0	0	0	32	40	37	34	129	108	152
2.5	0	0	0	0	0	0	0	0	112	67	67
0.0001 NQO	17	13	20	13	95	77	118	101	115	91	96
Expt 2B +S9 after 1 hr at 37°C											
0	5	2	5	C	56	48	39	51	109	142	85
0.01	4	8	4	3	41	42	46	29	144	112	61
0.1	6	5	4	6	31	38	48	41	177	152	129
0.5	4	2	4	3	31	38	36	45	155	136	134
1.0	1	0	1	1	29	36	23	32	122	128	105
2.5	0	0	0	0	1	1	0	0	113	109	146
10 CP	9	14	5	4	46	43	39	45	150	106	145
Expt 2C +S9 after 4 hrs at 37°C											
0	4	4	1	7	43	50	34	42	140	114	135
0.01	5	3	4	5	45	39	46	33	184	156	156
0.1	4	5	2	8	42	34	31	46	170	121	150
0.5	7	5	6	8	40	43	44	30	119	121	131
1.0	0	2	2	6	34	28	30	26	120	137	142
2.5	0	0	0	0	0	1	1	1	136	78	161
10 CP	23	23	24	25	214	227	221	230	78	61	72

C = contaminated

E = plate lost due to experimental error

APPENDIX II

CYTOGENETICS REPORT

Title: Toxicity studies with Mining Chemicals: In vitro chromosome studies with sodium isopropyl xanthate (SIX).

Responsible Practitioners: G. Hodson-Walker.

Work done: The cytogenetic effects of SIX was investigated in monolayer slide cultures of rat liver (RL_4) cells.

RESULTS

Initially cultures of RL_4 cells were exposed to 1.0, 2.0 or 4.0 µg/ml of SIX. The only finding of note was a substantial increase in the frequency of chromatid gaps and a single cell containing 3 exchange figures in cultures exposed to 1.0 µg/ml (Tables 2.1a and 2.1b). Cultures exposed to 2.0 or 4.0 µg/ml showed no significant increase in the incidence of chromosome damage.

A second experiment was then carried out in which cultures of RL_4 cells were exposed to 0.25, 0.5 or 1.0 µg/ml of SIX. In this study there was no significant increase in the incidence of chromosome damage in any of the cultures exposed to SIX (Tables 2.2a and 2.2b), but due to a low yield of metaphases (i.e. <300 per dose level) a third assay was carried out.

In the third study of identical design to the first, the frequency of chromosome damage did not differ significantly from the control values (Tables 2.3a and 2.3b).

In all three studies cultures exposed to the positive control substance, DMBA, showed a marked increase in chromosome damage.

G. Hodson-Walker

G. Hodson-Walker
Responsible Practioner
Date: 1.5.81.

Table 2.1a - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA)

Compound	Conc. µg/ml	No. of cultures	No. of cells analysed	% cells showing				Frequency per cell of					
				Polyploidy (1)	Chromatid gaps (2)*	Multiple chromatid damage (3)	Chromatid aberrations (4)*	Chromosome aberrations (5)	Chromatid gaps *	Chromatid breaks (6)*	Chromatid exchanges	Chromosome breaks	Chromosome exchanges (7)
Sodium isopropyl xanthate	0	3	254	1.6	2.0	0	0	0	0.020	0	0	0	0
	1	3	300	2.0	12.7	0	0.7	1.0	0.167	0.003	0.010	0.017	0
	2	3	300	1.3	2.3	0	0	0.3	0.023	0	0	0.003	0
	4	3	149	0.7	0.7	0	0.7	0	0.007	0.007	0	0	0
DMBA	1	2	132	2.3	28.0	5.3	5.3	0.8	0	0	0.14	0.008	0

* Cells with multiple chromatid damage excluded
(1) Polyploidy + endoreduplication (2) Gaps + iso-gaps (3) Gaps + breaks exchanges or any combination
(4) Breaks + single fragments + exchange figures (5) Acentric fragments + dicentrics + rings + translocations
(6) Single fragments + chromatid breaks (7) Dicentrics + translocations + rings.

Table 2.1b - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-diemthylbenzanthracene (DMBA) [Raw data]

Compound	Conc. µg/ml	CYT/ 307	No. of cells analysed	Number of aberrations per culture										
				Poly-ploidy	Endo-reduplication	Chromatid gaps	Iso-gaps	Chromatid breaks	Single fragments	Acentric fragments	Exchange figures	MCB	MCA	Dicentrics
Sodium isopropyl xanthate	0	007	100	2		3								
	0	010	100	2		1								
	0	013	54			1								
	1	005	100	3		18			1					
	1	009	100		1	16	1			2				
	1	014	100	2		15				3	3			
	2	002	100	2		5								
	2	008	100			1				1				
	2	011	100	2			1							
	4	001	3											
	4	013	46	1		1			1					
	4	012	100											
DMBA	1	004	32	1		11					3			
	1	006	100	2		77	3		1	1	16			

CYT = cytogenetic code number
MCB = multiple chromatid breaks
MCA = multiple chromatid aberrations

Table 2.2a - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA)

Compound	Conc. µg/ml	No. of cultures	No. of cells analysed	% cells showing					Frequency per cell of				
				Polyploidy (1)	Chromatid gaps (2)*	Multiple chromatid damage (3)	Chromatid aberrations (4)*	Chromosome aberrations (5)	Chromatid gaps *	Chromatid breaks (6)*	Chromatid exchanges	Chromosome breaks	Chromosome exchanges (7)
Sodium isopropyl xanthate	0	3	234	0.4	3.8	0	1.7	0.4	0.047	0.004	0.017	0.004	0
	0.25	3	273	0	0	0	0	0	0	0	0	0	0
	0.5	3	272	0.7	1.8	0	0.4	0.4	0.026	0.004	0	0.004	0
	1.0	3	108	0.9	4.6	0	0	1.9	0.074	0	0	0.019	0
DMBA	1	2	114	0	12.3	1.8	4.4	0	0.184	0.018	0.035	0	0

* Cells with multiple chromatid damage excluded
(1) Polyploidy + Endureduplication (2) Gaps + iso-gaps (3) Gaps + breaks + exchanges or any combination
(4) Breaks + single fragments + exchange figures (5) Acentric fragments + dicentrics + rings + translocations
(6) Single fragments + chromatid breaks (7) Dicentrics + translocations + rings

Table 2.2b - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA) [Raw data]

Compound	Conc. µg/ml	CYT/ 348	No. of cells analysed	Number of aberrations per culture										
				Poly-ploidy	Endo-redup-lication	Chromatid gaps	Iso-gaps	Chromatid breaks	Single fragments	Acentric fragments	Exchange figures	MCB	MCA	Dicen-trics
Sodium isopropyl xanthate	0	004	100			11		1		1	4			
	0	008	100	1										
	0	014	34											
	0.25	001	100											
	0.25	006	100											
	0.25	007	73											
	0.5	002	72							1				
	0.5	003	100	1										
	0.5	012	100	1		6								
	1.0	009	100	1		1	1	1		2				
	1.0	010	6											
	1.0	013	2			7								
DMBA	1	005	72			1		1	1		4			
	1	011	42			20		1					1	

CYT = cytogenetic code number
MCB = multiple chromatid breaks
MCA = multiple chromatid aberrations

Table 2.3a - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA)

Compound	Conc. µg/ml	No. of cultures	No. of cells analysed	% cells showing					Frequency per cell of				
				Polyploidy (1)	Chromatid gaps (2)*	Multiple chromatid damage (3)	Chromatid aberrations (4)*	Chromosome aberrations (5)	Chromatid gaps *	Chromatid breaks (6)*	Chromatid exchanges	Chromosome breaks	Chromosome exchanges (7)
Sodium isopropyl xanthate	0	3	300	1.7	0.3	0	0	0	0.003	0	0	0	0
	1	3	300	0	0	0	0	0	0	0	0	0	0
	2	3	300	1.3	0.3	0	0.7	0	0.003	0.003	0.003	0	0
	4	3	279	1.1	0.7	0	0	0	0.007	0	0	0	0
DMBA	1	2	70	0	5.7	0	7.1	0	0.057	0	0.086	0	0

* Cells with multiple chromatid damage excluded
(1) Polyploidy Endoreduplication (2) Gaps + iso-gaps (3) Gaps + breaks + exchanges or any combination
(4) Breaks + single fragments + exchange figures (5) Acentric fragments + dicentrics + rings + translocations
(6) Single fragments + chromatid breaks (7) Dicentrics + translocations + rings.

Table 2.3b - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA) [Raw data]

Compound	Conc. µg/ml	CYT/ 355	No. of cells analysed	Number of aberrations per culture										
				Poly-ploidy	Endo-redup-lication	Chromatid gaps	Iso-gaps	Chromatid breaks	Single fragments	Acentric fragments	Exchange figures	MCB	MCA	Dicen-trics
Sodium isopropyl xanthate	0	004	100	3										
	0	009	100	1		1								
	0	011	100	1										
	1	001	100											
	1	006	100											
	1	013	100											
	2	003	100			1			1		1			
	2	008	100	1										
	2	014	100	3										
	4	005	100	2		2								
	4	007	100											
	4	012	79	1										
DMBA	1	002	43			2					4			
	1	010	27			2					2			

CYT = cytogenetic code number
MCB = multiple chromatid breaks
MCA = multiple chromatid aberrations

APPENDIX 3

Compound Control and Formulation Chemistry Report

Title of Main Report:	Toxicity studies with Mining Chemicals: Short-term mutagenicity studies with sodium isopropyl xanthate.
Author:	[redacted]
Summary:	Data concerning test and control substances and their formulations are reported.

Test substance

Data describing the test substance used in this study are tabulated below:

Name	:	Sodium isopropyl xanthate
Source	:	Shell Santiago, Chile
CAS Ref. No.	:	[140-93-2]
Code No.	:	SF 113
Batch No.	:	SECADO 1734
Appearance	:	Yellowish pellets
Purity	:	Approximately 80-90% by mass
Date released	:	22nd June 1979, 17th June 1980 and 20th November 1980

The identity of the test substance was proved by infra-red spectrometry. The same technique was used to demonstrate the stability before re-release.

Formulation of test substance

The test substance was formulated as solutions in sterile distilled water. The concentrations ranged from 400 mg/ml to 0.001 mg/ml. These were made by dissolving a known mass of test substance in sterile distilled water and diluting as required.

Stability of formulations of test substance

Sodium isopropyl xanthate is produced by the interaction of isopropanol and carbon disulphide in aqueous sodium hydroxide. Xanthates are hydrolysed in aqueous solution, but this is not a rapid reaction in water at ambient temperature. The major use of alkali metal xanthates is as collectors in the flotation of metallic sulphide ores, which is done in aqueous solution.

On the basis of an examination of the chemistry and usages of xanthates, it was judged that aqueous solutions of sodium isopropyl xanthate would be stable for one working day.

Control substances

The control substances available for use in this study are shown below:

Control Substances	Source	Batch No.	Vehicle	Concentration
Benzo(a)pyrene Benzo(a)pyrene	Koch-Light Koch-Light	62991 76096	Dimethyl Sulphoxide (DMSO)	0.25, 0.5 and 1 mg/ml
Cyclophosphamide	Koch-Light	74841	Water	25 mg/ml
Neutral Red	G. T. Gurr	-	Water	1 mg/ml
4-Nitro-quinoline-N-oxide	ICI Ltd. Central Toxicology Laboratory	3757-10	DMSO	0.01, 0.1 and 1 mg/ml
Sodium azide	Fisons	40	Water	1 mg/ml
7,12-dimethyl-benzanthracene	Eastman Kodak	A6B	DMSO	0.5 mg/ml

Data on which shelf lives of formulations of control substances were based are shown below. Stability studies were not necessarily carried out on the same batches as were used for this study, but are considered to be independent of the batch.

Formulation and stability of benz(a)pyrene

Benz(a)pyrene (also termed 1,2-benzopyrene or 3,4-benzopyrene) Fig. 1 is normally formulated either as a solution in acetone or in dimethyl sulphoxide (DMSO). The stability of benz(a)pyrene as a solution in acetone or DMSO has been determined. This was done by analysis of fresh and stored solutions of benz(a)pyrene by high performance liquid chromatography using the following analytical conditions:-

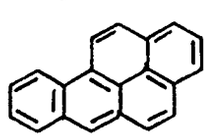
Column	-	0.25 m x ¼" O.D., 4 mm I.D. stainless steel
Packing	-	10 micron Spherisorb ODS 18
Detector	-	Cecil CE 212 Ultraviolet detector operating at 365 nm.
Solvent	-	80/20 (v/v) acetonitrile/distilled water (degassed)
Flow rate	-	1.6 ml/min.

The concentration of solutions used for stability studies and conditions of storage are summarised in the table.

Solvent	Concentration of Benz(a)pyrene (µg/ml)	Storage Conditions
DMSO	1000	Clear glass flask in the light @ ≃ +20°C
Acetone	50	Clear glass flask in the dark @ ≃ -18°C
Acetone	37.5	Clear glass flask in the dark @ ≃ -18°C
Acetone	12.5	Clear glass flask in the dark @ ≃ -18°C

The chromatograms obtained from fresh solutions were identical with those obtained from solutions stored for four weeks. This demonstrates that there is no appreciable decomposition when solutions of Benz(a)pyrene are stored as shown in the table. This is substantiated by results from in vitro mutagenicity testing, when solutions up to four weeks old retain their activity.

Fig. 1



Benz(a)pyrene,
1,2-benzopyrene
3,4-benzopyrene

Formulation and stability of cyclophosphamide

Cyclophosphamide is formulated as an aqueous solution. Such solutions decompose on storage. According to the British Pharmaceutical Codex, aqueous solutions of cyclophosphamide may be kept for a few hours at room temperature. All solutions of cyclophosphamide expire on the day of formulation.

Formulation and stability of neutral red

Neutral Red is formulated as an aqueous solution. The stability of an aqueous solution (1 mg/ml) of neutral red stored at room temperature (≈ 20°C) in the dark in a volumetric flask for 34 days has been determined. This was done by comparing a freshly prepared solution with a solution stored for 34 days, as above by a spectrophotometric technique.

The method used was as follows:-

0.5 ml of the fresh and stored solution were diluted to 250 ml with distilled water. The resulting dilute solutions were examined by spectrophotometry at wavelengths between 450 and 800 nm using water in the reference beam.

Both fresh and stored solutions of the neutral red gave similar spectra and were considered to be stable over this period.

This study demonstrated that a shelf life of 4 weeks can be assigned to aqueous solutions of neutral red.

This is substantiated by results from in vitro mutagenicity testing, when solutions up to 4 weeks old retain their activity.

Formulation and stability of 4-nitroquinoline-N-oxide

4-Nitroquinoline-N-oxide (NQO) is normally formulated as a solution in dimethyl sulphoxide (DMSO). The stability of NQO as a solution in DMSO has been determined. This was done by analysing fresh and stored solutions of NQO in DMSO (1 mg/ml) by high performance liquid chromatography using the following analytical conditions.

Column	-	0.25 x ¼" O.D., 4 mm I.D. stainless steel
Packing	-	10 micron Spherisorb ODS 18
Detector	-	Cecil CE 212 Ultraviolet detector operating at 365 nm.
Solvent	-	80/20 (v/v) acetonitrile/distilled water (degassed)
Flow rate	-	1.6 ml/min.

A solution of NQO in DMSO (1 mg/ml) was stored at room temperature (≃ 20°C) in a clear glass stoppered container for four weeks. A fresh solution was prepared, and both were analysed as described. The chromatograms obtained from the fresh and stored solutions were identical. This demonstrates that there is no appreciable decomposition when solutions of NQO in DMSO are stored as described. This is substantiated by results from in vitro mutagenicity testing, when solutions up to four weeks old retain their activity.

Formulation and stability of sodium azide

Sodium azide is formulated as an aqueous solution. The stability of an aqueous solution (0.25 mg/ml) of sodium azide stored in a dark glass bottle at room temperature (≃ 20°C) for 37 days has been determined. This was done by comparing a freshly prepared solution with a solution stored for 37 days, as above, by a colorimetric technique.

The method used was as follows:- A portion of the stored solution (1.0 ml) was mixed with a 0.5% aqueous solution of ferric sulphate (1.0 ml). The resulting red colour was measured spectrophotometrically using a 5 mm path length and a 2.5 mg/ml aqueous solution of ferric sulphate in the reference beam. A fresh solution of sodium azide in water (0.25 mg/ml) was analysed similarly.

Both fresh and stored solutions of sodium azide gave similar spectra (λ max 452 nm, 0.593 Absorbance for fresh; λ max 454 nm, 0.585 Absorbance for stored solutions). The sodium azide solution had therefore retained its strength during the 37 days' of storage.

This study demonstrated that a shelf life of 4 weeks can be assigned for aqueous solutions of sodium azide.

This is substantiated by results from in vitro mutagenicity testing, when solutions up to 4 weeks retain their activity.

Formulation and stability of 9,10-dimethyl-1,2-benzanthracene

9,10-Dimethyl-1,2-benzanthracene (DMBA) is normally formulated as a solution in dimethyl sulphoxide (DMSO). Studies of the stabilities of solutions containing 10 mg/ml and 0.5 mg/ml of DMBA in DMSO have been carried out using high performance liquid chromatography. The following analytical conditions were employed:-

Column	-	0.25 m x ¼" O.D., 4 mm I.D. stainless steel
Packing	-	10 micron Spherisorb ODS 18.
Detector	-	Cecil CE 212 Ultraviolet detector operating at 365 nm
Solvent	-	80/20 (v/v) acetonitrile/distilled water (degassed)
Flow rate	-	1.6 ml/min.

Solutions of DMBA in DMSO (10 and 0.5 mg/ml) were stored in stoppered clear glass vessels at room temperature (≃ 20°C) for up to five weeks. Fresh solutions were prepared and the old and fresh solutions were analysed as described. The chromatograms obtained from old and fresh solutions were identical, indicating that no decomposition had taken place.

When stored solutions of DMBA in DMSO were used as positive controls for <u>in vitro</u> mutagenicity testing, it was found that there had been a decrease in biological activity. There was thus a conflict between the results of chemical and biological analysis. The reason for this conflict was not resolved, but solutions of DMBA are not used beyond the date of formulation.

[redacted]

[redacted], B.A., Ph.D., M.R.S.C., C. Chem.
Responsible Practitioner
Date. 1st May 1981

QUALITY ASSURANCE STATEMENT

REPORT NUMBER: TLGR.80.156

EXPERIMENT NUMBER: IMX-1628

REPORT TITLE: Toxicity studies with Mining Chemicals: *In vitro* genotoxicity studies with sodium isopropyl xanthate.

STUDY DIRECTOR: T.M. Brooks

The procedures that were used in this study have been inspected and this report has been audited to ensure that it accurately describes the methods used and that the reported results accurately reflect the raw data of the study.

Date of inspection or audit.	Principal Subject	Date of written QA report to management
25 to 29. 2.80	Microbial assay	4. 3.80
14 to 16. 5.80	Chromosome assay	4. 6.80
29. 4.80	Chemical formulation	7. 5.80
22. 4.81	Study report	22. 4.81

[redacted]

QUALITY ASSURANCE INSPECTOR.

[redacted]

TLGR.80.156

TOXICITY STUDIES WITH MINING CHEMICALS: IN VITRO GENOTOXICITY STUDIES WITH SODIUM ISOPROPYL XANTHATE

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GROUP RESEARCH REPORT

TLGR.0013.77

TOXICITY OF WL 43775 INTERMEDIATES: ACUTE TOXICITY, SKIN AND EYE IRRITANCY AND SKIN SENSITIZATION POTENTIAL OF M-BROMOBENZALDEHYDE.

SICC/CAMK Budget Ref: 50070712

[redacted]

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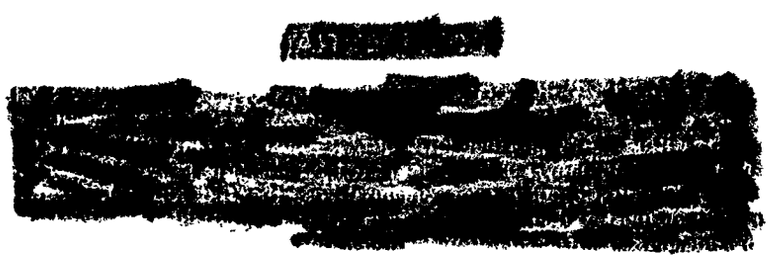

SHELL TOXICOLOGY LABORATORY (TUNSTALL)

Group Research Report TLGR.0013.77

Title: Toxicity of WL 43775 intermediates: Acute toxicity skin and eye irritancy and skin sensitization potential of m-bromobenzaldehyde.

Author: [redacted]

Reviewed by: [redacted].

Work done by: Experimental Toxicology Division of Shell Toxicology Laboratory (Tunstall).

Object: To determine the acute toxicity, skin and eye irritancy and skin sensitization potential of m-bromobenzaldehyde.

Summary:

1. The single dose acute oral LD_{50} value of m-bromobenzaldehyde administered to rats as a 10% w/v aqueous suspension was greater than 500 mg/kg.

2. The single dose acute percutaneous LD_{50} value of m-bromobenzaldehyde administered to rats as a 10% w/v aqueous suspension was greater than 300 mg/kg.

3. One 24 hour application of undiluted m-bromobenzaldehyde to occluded rabbit skin was moderately irritant.

4. Undiluted m-bromobenzaldehyde was mildly to moderately irritant to rabbit eyes.

5. m-Bromobenzaldehyde was found to elicit a moderate skin sensitization response in guinea-pigs.

[redacted]

[redacted],M.A.,Ph.D.,B.Sc.,B.V.Sc.,M.R.C.V.S.
Director, Shell Toxicology Laboratory (Tunstall)

Date: March, 1977.

INTRODUCTION

m-Bromobenzaldehyde is an intermediate used in the manufacture of WL 43775. The present studies were carried out in order to allow guidance to be given on the safe handling of this material.

EXPERIMENTAL

Materials

m-Bromobenzaldehyde was supplied to Shell Toxicology Laboratory (Tunstall) by Koninklijke/Shell-Laboratorium, Amsterdam, as a liquid.

Animals

Species	Strain/Breed	Source
Rat	CD	Charles River (U.K.) Ltd., Manston, Kent.
Rabbit	New Zealand White	Ranch Rabbits, Crawley, Sussex.
Guinea-pig	'P' Strain	Shell Toxicology Laboratory (Tunstall), Breeding Unit.

METHODS

The experimental details for each of the procedures referred to below, are given in the Appendix.

1. Acute oral toxicity

m-Bromobenzaldehyde was administered to rats as a 10% w/v dilution in 0.5% w/v aqueous carboxymethyl cellulose (CMC).

2. Acute percutaneous toxicity

The single dose acute percutaneous LD_{50} to rats of m-bromobenzaldehyde administered as a 10% w/v dilution in 0.5% w/v aqueous CMC was determined according to the method described by Noakes and Sanderson[1].

3. Primary irritation of the skin

The occluded patch test of Draize[2] was used to assess the skin irritation potential of undiluted m-bromobenzaldehyde to abraded and intact rabbit skin.

4. Eye irritation

The method used to determine the eye irritancy of undiluted m-bromobenzaldehyde to rabbits was based on that described in the U.S. Federal Register[3].

5. Skin sensitization

The guinea-pig maximization test as described by Magnusson and Kligman[4] was used to assess the skin sensitization potential of m-bromobenzaldehyde.

The concentrations of the test material used for induction and challenge are tabulated below:

Concentration % w/v m-bromobenzaldehyde in corn oil		
Intradermal induction	Topical induction	Topical challenge
0.1	25	15

RESULTS AND DISCUSSION

Acute toxicity (Tables 1a and 1b)

The acute oral LD_{50} value of m-bromobenzaldehyde was found to be greater than 500 mg/kg. No signs of toxicity were noted. One male rat dosed at 250 mg/kg was found dead on day 2, but this death was most probably un-related to the chemical.

The acute percutaneous LD_{50} value of m-bromobenzaldehyde was found to be greater than 300 mg/kg; this was the largest amount that could be dosed. There were no signs of toxicity or deaths at this level.

Primary skin irritation (Table 2)

One 24 hour application of undiluted m-bromobenzaldehyde to both abraded and intact occluded rabbit skin caused a mild erythema and oedema in females and moderate erythema and oedema in males. In one male rabbit a superficial burn was evident at 48 hours and the skin of this animal was necrotic at 7 days. Signs of mild skin irritation were still discernible at 7 days in four of the other test animals.

Eye irritation (Table 3)

Undiluted m-bromobenzaldehyde was mildly to moderately irritant to rabbit eyes causing a mild transient conjunctivitis in three animals and a more persistent conjunctivitis accompanied by minimal corneal opacity in another test rabbit. There were no signs of eye irritation in any of the animals 7 days after installation of m-bromobenzaldehyde.

Skin sensitization (Table 4)

14 out of the 20 test guinea-pigs previously exposed to m-bromobenzaldehyde developed a skin sensitization reaction. The degree of response would indicate that m-bromobenzaldehyde has a moderate skin sensitizing potential in guinea-pigs.

[redacted]

[redacted] B.Sc., M.Sc.

REFERENCES

1. Noakes, D. N. and Sanderson, D. M., (1969).
A method for determining the dermal toxicity of pesticides.
Br. J. industr. Med., 26, 59-64.

2. Draize, J. H., (1959).
'Dermal Toxicity' in "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics".
Association of Food and Drug Officials of the United States of America.

3. Federal Register, 28, (110). 6.6.1963.
para. 191.12. Test for eye irritants.

4. Magnusson, B. and Kligman, A. M., (1969).
The identification of contact allergens by animal assay.
The guinea-pig maximization test.
J. Invest. Derm., 52, 268-276.

Table 1a - Acute oral toxicity to rats of m-Bromobenzaldehyde administered as a 10% w/v dilution in 0.5% w/v aqueous CMC

Dosage mg/kg	Cumulative mortality		
	Males	Females	Total
250	1/2*	0/2	1/4
500	0/2	0/2	0/4

*Animal died on day 2 after dosing

Signs of toxicity : None observed

LD_{50} value : >500 mg/kg

Table 1b - Acute percutaneous toxicity to rats of m-Bromobenzyaldehyde administered as a 10% w/v dilution in 0.5% w/v aqueous CMC

Dosage mg/kg	Cumulative mortality		
	Males	Females	Total
300	0/2	0/2	0/4

Signs of toxicity : None observed

LD_{50} value : >300 mg/kg

Table 2 - Primary skin irritation of occluded rabbit skin after single application of undiluted m-Bromobenzaldehyde

Animal No.	Sex		Response	24 hours	48 hours	72 hours	7 days
1960	F	Abraded patch	Erythema	0-1	0	0	0
			Oedema	0-1	0	0	0
1961	F	Non-abraded patch	Erythema	1	1	0-1	0-1
			Oedema	0-1	0	0	0
4164	F	Abraded patch	Erythema	1	0-1	0	0
			Oedema	0-1	0	0	0
1962	F	Non-abraded patch	Erythema	1	0	0	0
			Oedema	0-1	0	0	0
4165	M	Abraded patch	Erythema	2	2*	2*	2*
			Oedema	2	2	1	2
4166	M	Non-abraded patch	Erythema	2	1-2	1	0-1
			Oedema	2	1	1	0
4167	M	Abraded patch	Erythema	2	1-2	0-1	0-1
			Oedema	2	1	0	0
4168	M	Non-abraded patch	Erythema	2	2	1	0-1
			Oedema	2	1	0	0

Scale: Readings 0 = No erythema to 4 = Beet redness *Chemical burn

Readings 0 = No oedema to 4 = Severe oedema

Table 3 - EYE IRRITATION

Compound: m-Bromobenzaldehyde (undiluted)

Dose: 0.2 ml in Right eye

		Rabbit No. Female 4184						Rabbit No. Male 4185						Rabbit No. Female 4249						Rabbit No. Male 4250					
With or without irrigation		Without						Without						With						With					
Initial effect		Pain						Pain						Pain						Pain					
Time after dosing		1h	24 h	48 h	72 h	7 days	days	1h	24 h	48 h	72 h	7 days	days	1h	24 h	48 h	72 h	7 days	days	1h	24 h	48 h	72 h	7 days	days
	Scale																								
CONJUNCTIVA																									
redness	0-3	0-1	0	0	0	0	-	1	0	0	0	0	-	1	1	0-1	0	0	-	1	2	1	0-1	0	-
chemosis	0-4	0	0	0	0	0	-	1	0	0	0	0	-	0-1	0	0	0	0	-	0-1	0	0	0	0	-
discharge	0-3	0	0	0	0	0	-	0	0	0	0	0	-	0-1	0	0	0	0	-	0-1	0-1	0	0	0	-
CORNEA																									
opacity	0-4	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-	1	1	0-1	0-1	0	-
area	0-4	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-	4	4	4	2	0	-
IRIS	0-2	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-

Comments: Skin around eye red at 1 hour. Male 4250 had darkened eye at 1 hour.

Table 4 - Skin sensitization reaction in guinea-pigs exposed to m-Bromobenzaldehyde

Animal number and sex	Skin response after challenge procedure		
	Immediate	24 hours	48 hours
Male 1	Trace	+ (S)	+ (S)
Male 2	Trace	Trace	-
Male 3	Trace	Trace	-
Male 4	-	-	-
Male 5	Trace	-	-
Female 1	-	-	-
Female 2	Trace	-	-
Female 3	Trace	-	-
Female 4	+	+ (S)	+ (S)
Female 5	-	-	-
Male 6	Trace	+	Trace
Male 7	-	-	-
Male 8	Trace	+ (S)	+ (S)
Male 9	Trace	+ (S)	+ (S)
Male 10	+	++	+
Female 6	-	Trace	Trace
Female 7	-	-	-
Female 8	Trace	+ (S)	+ (S)
Female 9	Trace	+	Trace
Female 10	+	+ (S)	+ (S)
CONTROLS			
Male 1	-	-	-
Male 2	-	-	-
Male 3	-	-	-
Male 4	-	-	-
Male 5	-	-	-
Female 1	-	-	-
Female 2	-	-	-
Female 3	-	-	-
Female 4	-	-	-
Female 5	-	-	-

Key: - No reaction
Trace Mild erythema
+ Moderate erythema
++ Severe erythema
(S) Scabbing

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APPENDIX

ACUTE ORAL TOXICITY

Two rats of each sex, age approximately 12 weeks, were used at each dose level. Three animals of one sex were housed in each cage. The animals were weighed, fasted overnight and the calculated dose of test material administered by intraoesophageal intubation using a ball-point needle fitted to a syringe. After dosing, food and water were available ad libitum throughout a 9 day observation period.

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ACUTE PERCUTANEOUS TOXICITY

The method used was the same as that described by Noakes and Sanderson for pesticides.

Two rats of each sex, aged 12-13 weeks, were used at one dose level. The test material was placed onto the shorn dorso-lumbar skin and bandaged into contact with the skin using an impermeable dressing of aluminium foil and waterproof plaster. The rats were housed individually over the 24 hours exposure period during which time the animals were deprived of food but allowed water ad libitum.

After 24 hours the dressings were removed and the exposed area was washed with a tepid dilute detergent solution. The rats were then housed in cages of three of one sex and observed for signs of intoxication during the following 9 days.

Noakes, D. N. and Sanderson, D. M., (1969).
A method for determining toxicity of pesticides.
Br. J. industr. Med., 26, 59-64.

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PRIMARY IRRITATION OF THE SKIN

The method used was that described by Draize.

Primary irritation of the abraded and intact skin of each of four male and four female rabbits was measured. The dorsal hair between the shoulders and the hindquarters was closely shorn by means of electric clippers. A 2 x 2 cm area of the shorn skin was abraded using a fine hypodermic needle. Injuries were deep enough to disturb the stratum corneum but not sufficiently deep to cause bleeding. 2 x 2 cm lint patches were applied to the abraded and intact skin and 0.5 ml test material was applied to each. The patches were covered by an occlusive polyethylene film which was secured in position by means of an elastic adhesive bandage (3" Poroplast). The patches were left in place for 24 hours.

Reactions were assessed visually for the degree of erythema and oedema as shown in the table below. Seven days after the application of the test material a final visual assessment was made.

No erythema	=	0	No oedema	=	0
Pale pink	=	1	Soft skin	=	1
Redness	=	2	Oedema	=	2
Severe redness	=	3	More definite oedema	=	3
Beet redness	=	4	Severe oedema	=	4

Draize, J. H., (1969).
'Dermal Toxicity' in "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics".
Association of Food and Drug Officials of the United States of America.

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EYE IRRITATION

The method used was based on that described in the U.S. Federal Register.

One male and one female albino rabbits were used.

0.2 ml of the test compound was instilled into the conjunctival sac of one eye of each rabbit; the untreated eye served as a control. A visual assessment of irritancy was made 30 minutes after application and again 1, 2, 3 and 7 days after application, thence every 4 days until eye irritancy was no longer observed.

CORNEA

No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling or normal luster), details of iris clearly visible	(1)*
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Complete corneal opacity, iris not discernible	4

IRIS

Normal	0
Markedly deepended folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	(1)*
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

CONJUNCTIVAE

Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	(2)*
Diffuse beefy red	3
CHEMOSIS	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	(2)*
Swelling with lids about half closed	3
Swelling with lids more than half closed	4
DISCHARGE	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

*Bracketed figures indicate lowest grades considered positive under Section 191.12 of the Federal Hazardous Substances Labelling Act Regulations.

Federal Register, 28, (110), 6.6.1963.
para. 191.12. Test for eye irritants.

SHELL TOXICOLOGY LABORATORY (TUNSTALL)

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SKIN SENSITIZATION

The guinea-pig maximization procedure of Magnusson and Kligman, was used to assess the skin sensitizing potential of the test material.

A preliminary screen was carried out to determine the concentrations of test material to be used for intradermal induction, topical induction and topical challenge. Two male and two female guinea-pigs were used for each test concentration.

Groups of ten male and ten female guinea-pigs were used for the test and a further five males and five females as controls.

Induction was accomplished in two stages:-

(i) Intradermal injection

Two rows of three injections were made: one on each side of the midline in the shorn skin of the shoulder region as follows:-

Test animals	Controls
2 x 0.1 ml Freund's complete adjuvant*	FCA
2 x 0.1 ml Test material in corn oil	Corn oil
2 x 0.1 ml Test material in 50:50 FCA/corn oil	50:50 FCA/corn oil

The injection sites were just within the boundary of a 4 x 4 cm shaved area.

*Freund's adjuvant (complete) prepared by Difco Laboratories, Detroit, 1, Michigan, U.S.A.

(ii) Topical application

One week after the intradermal injections the same area was clipped free from hair.

A 4 x 4 cm patch of Whatman No. 3 mm filter paper was soaked in a solution of the test material, placed over the injection sites of the experimental animals and covered by overlapping plastic adhesive tape (1½" Blenderm). This in turn was firmly secured by an elastic adhesive bandage (3" Poroplast). The dressing was left in place for 48 hours.

Challenge procedure

The challenge procedure was carried out 2 weeks after topical induction. Challenge was accomplished by topical application of the challenge solution of the test material to the flank of both test and control groups of animals.

Hair was removed from a 3 x 3 cm area on the flank by clipping and then shaving with an electric razor. A 2½ x 2½ cm patch of Whatman No. 3 mm filter paper was soaked in the challenge solution and placed over the shaved area. This was then covered by overlapping adhesive tape (1½" Blenderm) which was in turn firmly secured by an elastic adhesive bandage (3" Poroplast). The patch was left in place for 24 hours and examination of the challenge site was made immediately 24 and 48 hours after removal of the dressing. Three hours before the 24 hour reading the treated skin was closely shaved by means of an electric razor.

Magnusson, B. and Kligman, A. M., (1969).
The identification of contact allergens by animal assay.
The guinea-pig maximization test.
J. Invest. Derm., 52, 268-276.

TLGR.0013.77

TOXICITY OF WL 43775 INTERMEDIATES: ACUTE TOXICITY, SKIN AND EYE IRRITANCY AND SKIN SENSITIZATION POTENTIAL OF M-BROMOBENZALDEHYDE.

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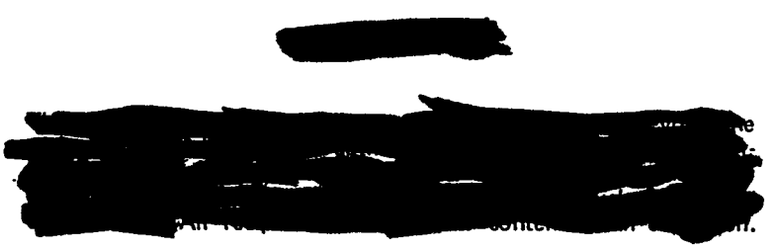
GROUP RESEARCH REPORT

TLGR.80.156

TOXICITY STUDIES WITH MINING CHEMICALS: IN VITRO GENOTOXICITY STUDIES WITH SODIUM ISOPROPYL XANTHATE

SICC/CIMS Budget Ref: 50070695



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SHELL TOXICOLOGY LABORATORY (TUNSTALL)

[redacted] [redacted]

Shell Toxicology Laboratory (Tunstall)

Group Research Report TLGR.80.156 Experiment Number 1MX-1628

Title: Toxicity studies with Mining Chemicals: In vitro genotoxicity studies with sodium isopropyl xanthate.

Introduction: This report describes the results of a series of in vitro tests to investigate the genotoxicity of sodium isopropyl xanthate. The assays include [redacted] agar overlay bacterial tests, a liquid culture assay for mitotic gene conversion in yeast and a cytogenetic study in cultured rat liver cells.

Date study started: 23rd July, 1979

Study Director: [redacted]

Authors: [redacted]

Responsible Practitioners: [redacted] Microbiologist; Technician (Cytogenetics); Formulation Chemist; Technician (Formulation); Compound Controller

Reviewer: [redacted]

Summary: The mutagenic activity of sodium isopropyl xanthate was investigated in agar layer cultures of Salmonella typhimurium and Escherichia coli bacterial tester strains and in liquid cultures of the yeast, Saccharomyces cerevisiae. Assays were performed both in the presence and absence of S9 microsomal fraction obtained from a liver homogenate from rats pre-treated with Aroclor. Monolayer slide cultures of rat liver (RL4) cells were cultured for 24 hours in culture medium containing sodium isopropyl xanthate; metaphase cells were analysed for structural chromosome aberrations.

The results indicate that sodium isopropyl xanthate did not induce mutation in bacteria, gene conversion in yeast or

chromosome damage in rat liver cells under the conditions of the assays described.

Microscope slide preparations of RL_4 cells are stored in the Chemical Mutagenesis Slide Archive, the raw data from all studies and the final report are stored in the Record, Shell Toxicology Laboratory (Tunstall).

[redacted], B.V.M.S., M.R.C.V.S., D.V.M., Ph.D., F.R.C. Path., F.I. Biol.
Director, Shell Toxicology Laboratory (Tunstall)
Sittingbourne Research Centre, Sittingbourne,
Kent, ME9 8AG.

Date: May 1981

[redacted]

INDEX

PROCEDURES

The microorganisms and the procedures are described in STL SOP 28/01/001 and STL SOP 28/01/004. The microorganisms used were Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100, Escherichia coli WP_2 and WP_2 uvr A and Saccharomyces cerevisiae JD1.

The rat liver (RL_4) cell culture and procedures are described in SOP 28/01/003.

Methods

a) Bacterial mutation study

20 µl volumes of 0.01, 0.1, 1.0, 10 or 100 mg/ml solutions of sodium isopropyl xanthate in distilled water were added to top agar mix to give final amounts of 0.2, 2.0, 20, 200 or 2000 µg per plate in both the presence and absence of rat liver S9 fraction. The cultures were incubated at 37°C for 48 hours before the revertant colonies were counted.

An additional experiment was carried out to study the influence of sodium isopropyl xanthate on the activity of the monooxygenase system in the rat liver S9 fraction. The positive control compound benzo(a)pyrene was incorporated in a conventional agar assay with Aroclor-induced rat liver S9 fraction and either 200 or 2000 µg per plate of sodium isopropyl xanthate using S. typhimurium TA 98. The amounts of benzo(a)pyrene tested were 5, 10, 20 µg per plate. After incubation, the revertant colonies were counted and the influence of sodium isopropyl xanthate on the benzo(a)pyrene-induced reversion frequency was determined.

b) Saccharomyces gene conversion assay

Liquid suspension cultures were dosed with 20 µl (without S9 mix) or 25 µl (with S9 mix) of 1, 10, 50, 100 or 250 mg/ml solutions of sodium isopropyl xanthate in water to give final concentrations of 0.01, 0.1, 0.5, 1.0 or 2.5 mg/ml both with and without the incorporation of rat liver S9 fraction. After 1 h incubation without S9 fraction and after 1 h and 4 h incubation with S9 fraction, the cultures were seeded onto the appropriate culture media for the selection of revertant colonies. After 3 days incubation at 30°C the numbers of revertant colonies were counted.

c) Rat liver chromosome assay

RL_4 slide cultures were exposed to culture medium containing sodium isopropyl xanthate at final concentrations of 0.25, 0.5, 1.0, 2.0 or 4.0 µg/ml. After 24 hours the cultures were processed for chromosome analysis and, where possible, 100 cells analysed from each of three cultures per dose group.

Materials

Sodium isopropyl xanthate was obtained from Shell Santiago, Chile (Batch No. Secado 1734) and prepared for use as solutions in sterile distilled water.

Benzo(a)pyrene, Batch No. KL 62991, was obtained from Koch-Light Laboratories and prepared as 0.25, 0.5 and 1.0 mg/ml solutions in dimethyl sulphoxide (DMSO).

Cyclophosphamide, Batch No. 74841, was obtained from Koch-Light Laboratories and prepared as a 25 mg/ml solution in sterile distilled water.

Neutral red was obtained from G.T. Gurr Ltd., London and prepared as as 1 mg/ml solution in water.

4-Nitroquinoline-N-oxide, Batch No. 3757-10, was a gift from Dr. J. Ashby, ICI Ltd., CTL, Alderley Edge, Cheshire and prepared as 0.01, 0.1 and 1 mg/ml solutions in DMSO.

Sodium azide, Batch No. 40, was supplied by Fisons Laboratory Equipment, Loughborough, Leics., and prepared as a 1 mg/ml solution in distilled water.

7,12-Dimethylbenzanthracene, Batch No. A6B, was supplied by Eastman-Kodak Co., Kirby, Liverpool, and prepared for use as a 0.5 mg/ml solution in DMSO.

DISCUSSION

Solutions of sodium isopropyl xanthate in water were shown to be stable for at least 4 hours (Appendix 3), which was the maximum period between preparation of the formulations and their incorporation in the assay systems.

In the bacterial assays, sodium isopropyl xanthate did not induce reverse gene mutations in the Salmonella or Escherichia tester strains.

It was considered that under certain in vitro experimental conditions, sodium isopropyl xanthate may inhibit mono-oxygenase enzyme activity (D. Hutson, personal communication). In order to ascertain whether sodium isopropyl xanthate interfered with the activity of rat liver S9 microsomal enzymes in the microbial assays, the test compound was studied in a mutation experiment using benzo(a)pyrene. Amounts of 200 or 2000 µg per plate of sodium isopropyl xanthate were incorporated in the agar overlay together with standard S9 mix, Salmonella typhimurium TA 98 and benzo(a)pyrene. A reduction in the mutagenic activity of benzo(a)pyrene was observed on the addition of 2000 µg per plate sodium isopropyl xanthate but not with 200 µg per plate. The activity of the S9 fraction was therefore not affected by the inclusion of sodium isopropyl xanthate at amounts up to 200 µg per plate.

Studies with sodium isopropyl xanthate in Saccharomyces cerevisiae JD1 showed that the compound did not induce mitotic gene conversion.

Sodium isopropyl xanthate did not induce detectable chromosome damage in the rat liver chromosome assay.

CONCLUSION

Applications of sodium isopropyl xanthate at amounts up to 2000 µg per plate did not increase the reverse mutation rate of Escherichia coli WP_2 and WP_2 uvr A or Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100 in vitro in the presence or absence of a rat liver microsomal activation system.

Exposure of Saccharomyces cerevisiae JD1 to sodium isopropyl xanthate in vitro in liquid culture at concentrations up to 2.5 mg/ml did not result in any consistent increase in the rate of mitotic gene conversion either in the presence or absence of a rat liver microsomal activation system.

As there was no increase in the frequency of chromatid gaps, chromatid breaks or total chromatid aberrations in cultures exposed to sodium isopropyl xanthate it is concluded that the compound did not induce chromosome damage in cultured rat liver (RL_4) cells.

The results show that sodium isopropyl xanthate does not induce reverse gene mutation in bacteria, mitotic gene conversion in yeast or chromosome damage in cultured rat liver cells under the experimental conditions described.

[redacted] M.I. Biol.
Study Director
Date: 1/5/81.

[redacted] . Walker
[redacted]
Responsible Practitioner
Date: 5.5.81

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APPENDIX 1

MICROBIOLOGY REPORT

Title: [redacted] Toxicity studies with Mining Chemicals: In vitro microbial mutation studies with sodium isopropyl xanthate.

Responsible Practitioner: [redacted] T. M. Brooks

Work done:

The mutagenic activity of sodium isopropyl xanthate was investigated in agar layer cultures of Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100, Escherichia coli WP_2 and WP_2 uvr A and in liquid cultures of Saccharomyces cerevisiae JD1 both with and without the incorporation of a rat liver microsomal activation system.

The influence of sodium isopropyl xanthate was also studied on the mutation frequency of benzo(a)pyrene using Salmonella typhimurium TA 98 in the presence of rat liver S9 fraction.

Results

a) Bacterial mutation study (Tables 1.1a, 1.1b, 1.1c and 1.1d)

The addition of sodium isopropyl xanthate to agar layer cultures of Escherichia coli WP_2 and WP_2 uvr A and Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100 both with and without the incorporation of a rat liver microsomal fraction (S9) did not lead to an increase in the reverse mutation frequency in any of the strains. The amounts of sodium isopropyl xanthate tested were 0.2, 2.0, 20, 200 or 2000 µg per plate.

The activity of the S9 mix and of the strains TA 98, TA 100 and TA 1538 was monitored by treating cultures with a known positive control compound benzo(a)pyrene which requires metabolic activation before it is able to induce gene mutation. The sensitivity of TA 1537 was monitored by the indirect mutagen neutral red and the E. coli strains and TA 1535 were monitored by testing with the direct-acting mutagens 4-nitroquinoline-N-oxide and sodium azide respectively.

The addition of 2000 µg per plate sodium isopropyl xanthate to 5, 10 or 20 µg per plate benzo(a)pyrene in the presence of rat liver S9 fraction resulted in an inhibition in response of strain TA 98 to benzo(a)pyrene-mediated mutagenicity (Table 1.1d). This effect was not seen with the addition of 200 µg per plate sodium isopropyl xanthate.

b) Saccharomyces gene conversion assay (Tables 1.2a and 1.2b)

The addition of sodium isopropyl xanthate to liquid suspension cultures of Saccharomyces cerevisiae JD1 with or without the addition of a rat liver microsomal fraction did not induce a consistent increase in mitotic gene conversion. The concentrations of sodium isopropyl xanthate tested were 0.01, 0.1, 0.5, 1.0 and 2.5 mg/ml. Treatment with 4-nitroquinoline-N-oxide, a direct-acting mutagen, and cyclophosphamide, and indirect mutagen, was shown to induce mitotic gene conversion.

[redacted], M.I. Biol.
Responsible Practitioner
Date: 1/5/81

Table 1.1a - Relative reverse mutation rates in Escherichia coli WP_2 and WP_2 uvrA and Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100 after treatment with sodium isopropyl xanthate in the plate incorporated assay

Micro-organisms	Experiment Number	Sodium isopropyl xanthate µg per plate																		
		Without Microsomal Activation (-S9)									With Microsomal Activation (+S9)									
		0.2	2.0	20	200	2000	NaN_3[a] 20 µg	BP[b] 20 µg	NQO[c] 20 µg	NR[d] 20 µg	0.2	2.0	20	200	2000	NaN_3[a] 20 µg	BP[b] 20 µg	NQO[c] 20 µg	NR[d] 20 µg
E. coli WP_2	5	0.5	1.0	0.8	1.1	0.8	-	-	11.8*	-	1.0	1.0	1.0	1.4	0.7	-	-	1.5	-
	8	1.1	0.7	0.9	1.3	1.1	-	-	33.3*	-	1.0	1.0	1.0	1.1	0.7	-	-	1.5	-
E. coli WP_2 uvr A	1	1.0	0.8	1.0	1.2	0.9	-	-	6.8*	-	0.8	0.9	0.9	1.5	0.9	-	-	36.2*	-
	5	1.1	1.0	1.0	1.3	1.2	-	-	18.8*	-	1.0	1.2	1.0	1.1	0.6	-	-	27.9*	-
S. typhimurium TA 1535	2	1.0	0.9	1.1	1.0	0	73.6*	-	-	-	0.9	0.9	0.8	0.9	0.7	105.3*	-	-	-
	4	1.0	1.5	1.3	1.7	0.5	179.6*	-	-	-	0.7	0.9	1.3	1.5	1.0	78.1*	-	-	-
S. typhimurium TA 1537	8	0.9	1.3	0.9	1.2	0.4	-	-	-	2.3	0.9	0.9	0.6	1.0	0.9	-	-	-	19.0*
	9	1.4	0.8	1.6	1.5	0.3	-	-	-	1.8	1.0	1.4	1.2	1.1	1.0	-	-	-	25.4*
S. typhimurium TA 1538	2	1.2	0.8	0.9	0.8	0.3	-	1.3	-	-	0.7	0.8	0.9	0.8	0.2	-	3.2*	-	-
	3	0.8	0.7	1.2	1.3	0.4	-	0.8	-	-	1.4	1.3	1.2	0.9	0.1	-	4.2*	-	-
S. typhimurium TA 98	6	0.6	1.3	0.8	1.0	0.9	-	1.0	-	-	1.0	1.0	0.9	0.9	0.6	-	3.3*	-	-
	7	1.5	1.4	1.1	1.7	1.3	-	1.4	-	-	0.8	0.8	1.0	1.5	0.7	-	3.1*	-	-
S. typhimurium TA 100	4	0.8	0.8	1.0	1.1	0.4	-	1.2	-	-	1.1	1.0	1.1	1.2	0.4	-	2.7*	-	-
	5	1.1	1.1	1.0	0.8	0.7	-	0.7	-	-	1.2	0.9	1.1	1.1	0.3	-	3.0*		

Results are expressed as a ratio: $\frac{\text{Mean number of revertant colonies per treated plate}}{\text{Mean number of revertant colonies per control plate}}$

(a) Sodium azide
(b) Benzo(a)pyrene
(c) 4-Nitroquinoline-N-oxide
(d) Neutral red

* Reproducible values of 2.5 x control value or greater are considered to indicate a mutagenic response.

- Not tested

Table 1.1b - Mean number of revertants per plate after treatment of bacteria with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO), benzo(a)pyrene (BP), sodium azide (NaN_3) or neutral red (NR) in the plate incorporated assay

µg/plate	*Escherichia coli* WP_2			
	Experiment 5		Experiment 8	
	-S9	+S9	-S9	+S9
0	10.0 ± 5.1	11.3 ± 4.0	5.8 ± 2.2	6.5 ± 2.4
0.2	5.3 ± 3.1	10.8 ± 2.6	6.3 ± 2.5	6.3 ± 2.1
2	9.8 ± 5.0	11.3 ± 2.6	3.8 ± 2.2	6.3 ± 0.5
20	7.5 ± 4.4	11.5 ± 1.7	5.0 ± 1.4	6.3 ± 1.5
200	10.5 ± 1.9	15.8 ± 4.5	7.5 ± 2.9	7.3 ± 1.3
2000	7.8 ± 1.7	8.0 ± 6.8	6.3 ± 2.8	4.5 ± 2.6
20 NQO	117.8 ± 48.4	16.8 ± 3.5	193.0 ± 91.0	9.5 ± 1.7
µg/plate	*Escherichia coli* WP_2 *uvr A*			
	Experiment 1		Experiment 5	
	-S9	+S9	-S9	+S9
0	11.5 ± 3.3	19.3 ± 9.3	11.0 ± 1.6	17.8 ± 2.2
0.2	11.0 ± 4.2	15.8 ± 7.2	11.8 ± 2.2	18.3 ± 2.5
2	9.0 ± 5.4	17.3 ± 8.0	11.0 ± 4.4	22.0 ± 3.7
20	11.3 ± 2.2	16.5 ± 11.8	10.5 ± 4.4	17.0 ± 2.9
200	13.5 ± 5.2	29.3 ± 7.0	13.8 ± 0.5	19.8 ± 4.3
2000	10.8 ± 4.8	18.3 ± 5.3	13.3 ± 3.8	10.0 ± 3.9
20 NQO	78.5 ± 75.8	699.0 ± 146.9	207.3 ± 201.0	496.0 ± 117.4
µg/plate	*Salmonella typhimurium* TA 100			
	Experiment 4		Experiment 5	
	-S9	+S9	-S9	+S9
0	62.0 ± 16.5	65.8 ± 12.4	71.3 ± 12.3	74.3 ± 8.7
0.2	48.5 ± 9.8	75.0 ± 19.8	77.5 ± 19.9	85.8 ± 24.0
2	52.0 ± 1.8	63.0 ± 15.7	77.0 ± 19.9	69.0 ± 7.4
20	61.8 ± 5.7	74.0 ± 7.9	70.8 ± 6.8	85.3 ± 18.0
200	69.3 ± 4.3	79.3 ± 10.9	54.8 ± 10.0	80.0 ± 23.8
2000	22.5 ± 8.6	25.8 ± 12 1	49.5 ± 8.7	21.3 ± 13.6
20 BP	73.3 ± 11.3	179.3 ± 22.3	51.5 ± 9.1	222.3 ± 81.2

Table 1.1b Contd

µg/plate	Salmonella typhimurium TA 1535			
	Experiment 2		Experiment 4	
	-S9	+S9	-S9	+S9
0	10.8 ± 4.0	10.8 ± 2.8	5.5 ± 1.3	13.0 ± 2.4
0.2	10.8 ± 5.0	9.5 ± 2.6	5.5 ± 1.7	9.3 ± 1.9
2	9.3 ± 3.0	9.3 ± 6.3	8.3 ± 3.7	12.0 ± 2.9
20	12.3 ± 4.8	8.8 ± 6.8	7.3 ± 3.8	16.8 ± 6.4
200	10.8 ± 3.0	9.5 ± 7.0	9.5 ± 3.7	19.5 ± 8.6
2000	0.3 ± 0.5	7.5 ± 4.9	3.0 ± 0.8	12.8 ± 3.2
20 NaN_3	794.8 ± 81.6	1137.5 ± 128.1	988.0 ± 88.3	1015.0 ± 67.6

µg/plate	Salmonella typhimurium TA 1538			
	Experiment 2		Experiment 3	
	-S9	+S9	-S9	+S9
0	8.3 ± 1.7	41.3 ± 9.2	2.0 ± 1.6	12.3 ± 5.6
0.2	10.3 ± 1.9	30.8 ± 4.1	1.5 ± 2.4	17.0 ± 2.7
2	6.8 ± 2.6	34.8 ± 5.0	1.3 ± 0.5	16.0 ± 7.0
20	7.3 ± 3.0	37.0 ± 7.7	2.3 ± 1.5	15.0 ± 5.2
200	7.0 ± 1.8	32.0 ± 3.7	2.5 ± 1.3	10.5 ± 3.0
2000	2.5 ± 1.9	7.8 ± 2.2	0.8 ± 0.5	1.8 ± 1.5
20 BP	10.8 ± 3.8	131.5 ± 16.4	1.5 ± 0.6	51.3 ± 10.2

µg/plate	Salmonella typhimurium TA 98			
	Experiment 6		Experiment 7	
	-S9	+S9	-S9	+S9
0	5.3 ± 1.7	10.3 ± 4.3	8.3 ± 2.4	14.8 ± 4.3
0.2	3.3 ± 2.6	10.0 ± 1.4	12.3 ± 5.7	12.3 ± 3.2
2	7.0 ± 4.8	10.0 ± 2.7	11.8 ± 4.3	11.8 ± 1.0
20	4.5 ± 3.9	9.0 ± 2.9	9.3 ± 2.5	14.3 ± 4.8
200	5.5 ± 1.7	9.3 ± 2.2	14.0 ± 5.9	22.5 ± 6.1
2000	4.8 ± 4.3	5.8 ± 3.5	11.0 ± 4.1	10.3 ± 2.6
20 BP	5.5 ± 5.4	34.3 ± 11.8	11.3 ± 2.2	45.5 ± 6.4

Table 1.1b Contd

µg/plate	Salmonella typhimurium TA 1537			
	Experiment 8		Experiment 9	
	-S9	+S9	-S9	+S9
0	5.5 ± 2.1	7.8 ± 3.5	4.3 ± 2.2	6.5 ± 1.3
0.2	4.8 ± 2.5	6.8 ± 2.2	6.0 ± 2.4	6.8 ± 1.9
2	7.3 ± 2.6	7.3 ± 2.2	3.3 ± 1.9	9.0 ± 0.8
20	4.8 ± 1.5	4.3 ± 2.1	6.8 ± 1.7	7.8 ± 3.3
200	6.8 ± 3.0	7.8 ± 1.0	6.3 ± 1.7	7.3 ± 3.5
2000	2.3 ± 1.5	7.0 ± 2.4	1.3 ± 1.3	6.3 ± 2.9
20 NR	12.5 ± 6.6	148.3 ± 24.8	7.8 ± 1.0	165.0 ± 48.7

Table 1.1c - Number of revertants per plate after treatment of bacteria with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO), benzo(a)pyrene (BP), sodium azide (NaN_3) or neutral red (NR) in the plate incorporated assay - raw data

µg/plate	*Escherichia coli* WP_2															
	Experiment 5								Experiment 8							
	-S9				+S9				-S9				+S9			
0	5	10	8	17	8	9	11	17	7	8	3	5	8	9	5	4
0.2	5	8	7	1	8	9	13	13	6	3	7	9	9	6	4	6
2	3	15	10	11	10	9	15	11	5	3	1	6	6	6	7	6
20	2	11	11	6	11	14	11	10	5	4	4	7	5	8	5	7
200	13	11	9	9	9	18	18	18	7	4	8	11	7	7	9	6
2000	8	10	6	7	17	9	1	5	8	3	9	5	2	8	3	5
20 NQO	105	184	68	114	13	15	21	18	202	143	110	317	10	10	7	11

µg/plate	*Escherichia coli* WP_2 *uvr A*															
	Experiment 1								Experiment 5							
	-S9				+S9				-S9				+S9			
0	16	11	11	8	E	30	13	15	9	11	11	13	17	17	21	16
0.2	15	14	6	9	22	22	9	10	11	15	11	10	18	19	15	21
2	13	3	6	14	26	22	11	10	6	9	16	13	26	22	17	23
20	12	12	8	13	34	13	11	8	7	9	9	17	17	21	14	16
200	20	8	15	11	31	29	37	20	14	13	14	14	17	26	19	17
2000	5	13	9	16	25	18	12	18	17	10	10	16	5	14	12	9
20 NQO	36	192	45	41	883	581	580	752	51	100	180	498	587	568	329	500

µg/plate	*Salmonella typhimurium* TA 100															
	Experiment 4								Experiment 5							
	-S9				+S9				-S9				+S9			
0	50	50	85	63	76	67	72	48	72	71	86	56	66	84	79	68
0.2	57	57	40	40	53	64	88	95	98	91	63	58	110	102	60	71
2	50	53	51	54	60	74	42	76	53	69	89	97	74	60	66	76
20	65	68	56	58	69	66	83	78	81	67	67	68	75	74	112	80
200	72	70	72	63	90	66	75	86	42	52	60	65	115	66	75	64
2000	17	34	15	24	13	38	34	18	61	42	52	44	5	38	23	19
20 BP	71	62	89	71	181	156	171	209	42	63	47	54	316	233	118	222

Table 1.1c Contd

µg/plate	Salmonella typhimurium TA 1535															
	Experiment 2								Experiment 4							
	-S9				+S9				-S9				+S9			
0	13	15	6	9	12	9	8	14	6	5	7	4	15	10	15	12
0.2	12	11	16	4	12	9	6	11	3	7	6	6	8	12	8	9
2	6	13	10	8	3	18	8	8	13	8	8	4	12	13	8	15
20	19	8	12	10	17	11	6	1	4	11	4	10	15	15	11	26
200	15	8	10	10	9	13	16	0	7	14	6	11	12	31	14	21
2000	1	0	0	0	13	10	5	2	3	2	4	3	10	15	10	10
20 NaN_3	799	888	803	689	986	1090	1191	1283	1081	1016	870	985	951	1013	987	1109

µg/plate	Salmonella typhimurium TA 1538															
	Experiment 2								Experiment 3							
	-S9				+S9				-S9				+S9			
0	8	9	6	10	31	49	36	49	2	4	0	2	10	12	7	20
0.2	10	9	13	9	32	28	27	36	0	1	5	0	18	19	18	13
2	3	9	8	7	34	38	28	39	1	2	1	1	7	17	16	24
20	4	8	11	6	44	42	35	27	1	3	1	4	20	19	11	10
200	5	6	8	9	34	36	28	30	2	3	1	4	9	9	9	15
2000	5	3	1	1	10	5	9	7	0	1	1	1	4	1	1	1
20 BP	10	6	15	12	108	137	146	135	1	2	2	1	51	57	37	60

µg/plate	Salmonella typhimurium TA 98															
	Experiment 6								Experiment 7							
	-S9				+S9				-S9				+S9			
0	5	7	3	6	13	13	4	11	8	5	10	10	11	18	19	11
0.2	6	1	1	5	10	9	12	9	7	19	15	8	11	17	11	10
2	14	6	5	3	9	14	8	9	8	8	16	15	12	13	11	11
20	1	3	4	10	5	10	12	9	6	9	10	12	9	20	16	12
200	4	7	7	4	12	10	7	8	20	14	16	6	17	25	30	18
2000	2	2	4	11	2	4	7	10	13	5	12	14	10	14	9	8
20 BP	13	6	1	2	44	43	31	19	9	10	12	14	55	42	42	43

Table 1.1c Contd

µg/plate	Salmonella typhimurium TA 1537															
	Experiment 8								Experiment 9							
	-S9				+S9				-S9				+S9			
0	5	3	6	8	9	4	12	6	2	5	3	7	5	7	8	6
0.2	8	4	2	5	10	5	6	6	3	9	6	6	7	8	4	8
2	6	11	7	5	5	8	10	6	2	2	3	6	9	8	10	9
20	3	4	6	6	4	2	4	7	9	6	5	7	7	4	8	12
200	6	6	4	11	7	8	7	9	6	8	7	4	11	9	6	3
2000	4	3	1	1	5	5	10	8	1	3	1	0	10	6	6	3
20 NR	8	12	22	8	168	169	118	138	7	8	7	9	128	119	216	197

E = plate lost due to experimental error.

Table 1.1d - The influence of sodium isopropyl xanthate (SIX) on mixed function oxidase-mediated mutagenicity of benzo(a)pyrene using S. typhimurium TA 98

Benzo(a)pyrene µg/plate	Mean number of revertants per plate		Number of revertants per plate (raw data)	
	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 2000 µg per plate SIX	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 2000 µg per plate SIX
Experiment 1				
0	29.3 ± 4.7	13.3 ± 3.2	28 25 28 36	12 12 11 18
5	130.8 ± 13.0	90.0 ± 25.2	142 114 127 140	65 101 74 120
10	192.3 ± 30.4	108.0 ± 45.3	205 147 213 204	138 85 153 56
20	139.0 ± 31.4	32.8 ± 10.5	135 183 129 109	23 38 25 45
Experiment 2				
0	15.3 ± 2.9	10.0 ± 1.4	17 17 11 16	12 10 9 9
5	72.0 ± 3.9	44.8 ± 11.6	70 77 73 68	30 42 57 50
10	58.0 ± 22.1	30.0 ± 12.8	31 49 74 78	19 44 42 47
20	52.0 ± 14.7	21.3 ± 12.5	34 46 64 64	11 30 10 34
	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 200 µg per plate Six	With rat liver microsomal enzymes (S9)	With rat liver microsomal enzymes (S9) + 200 µg per plate Six
Experiment 3				
0	25.3 ± 5.7	23.3 ± 5.4	21 21 33	30 20 18
5	102.0 ± 24.2	119.8 ± 24.3	128 79 84	108 92 146
10	212.8 ± 18.5	284.8 ± 18.0	222 201 234	290 270 308
20	333.8 ± 50.8	263.5 ± 55.9	266 354 329	281 209 230

Table 1.2a - Mitotic gene conversion in liquid cultures of Saccharomyces cerevisiae JD1 after treatment with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO) or cyclophosphamide (CP) in the presence and absence of rat liver S9 fraction

mg compound per ml	Survivors x 10^4 (per plate)	HISTIDINE LOCUS			TRYPTOPHAN LOCUS		
		Revertants per plate	Revertants per 10^6 survivors	Ratio(1) over control	Revertants per plate	Revertants per 10^6 survivors	Ratio(1) over control
Experiment 1A 1 hr -S9 at room temperature							
0	206	3.3	1.6	-	17.5	8.5	-
0.01	117	1.3	1.1	1	6.5	5.6	1
0.1	177	4.0	2.3	1	19.7	11.1	1
0.5	182	1.5	0.8	1	10.5	5.8	1
1.0	168	1.3	0.8	1	15.8	9.4	1
2.5	176	0	0	-	6.3	3.6	1
0.001 NQO	5	60.8	1216.0	760*	259.3	5186.0	610*
Experiment 1B 1 hr +S9 at 37°C							
0	145	2.5	1.7	-	7.0	4.8	-
0.01	123	1.5	1.2	1	5.8	4.7	1
0.1	129	1.3	1.0	1	5.3	4.1	1
0.5	130	1.3	1.0	1	4.0	3.1	1
1.0	187	0.3	0.2	1	4.8	2.6	1
2.5	103	0	0	-	0.5	0.5	1
10 CP	119	2.5	2.1	1	11.3	9.5	2
Experiment 1C 4 hr +S9 at 37°C							
0	115	2.8	2.4	-	19.5	17.0	-
0.01	159	1.3	0.8	1	7.7	4.8	1
0.1	127	0.8	0.6	1	5.0	3.9	1
0.5	120	2.5	2.1	1	14.0	11.7	1
1.0	92	0.5	0.5	1	7.8	8.5	1
2.5	59	0	0	-	0	0	-
10 CP	106	22.0	20.8	9*	189.0	178.3	10*
Experiment 2A 1 hr -S9 at room temperature							
0	154	6.8	4.4	-	41.3	26.8	-
0.01	148	5.8	3.9	1	54.8	37.0	1
0.1	147	8.3	5.6	1	47.0	32.0	1
0.5	102	2.3	2.3	1	41.8	41.0	2
1.0	130	0	0	-	35.8	27.5	1
2.5	82	0	0	-	0	0	-
0.0001 NQO	101	15.8	15.6	4*	97.8	96.8	4*
Experiment 2B 1 hr +S9 at 37°C							
0	112	4.0	3.6	-	48.5	43.3	-
0.01	106	4.8	4.5	1	39.5	37.3	1
0.1	153	5.3	3.5	1	39.5	25.8	1
0.5	142	3.3	2.3	1	37.5	26.4	1
1.0	118	0.8	0.7	1	30.0	25.4	1
2.5	123	0	0	-	0.5	0.4	1
10 CP	134	8.0	6.0	2	43.3	32.3	1
Experiment 2C 4 hr +S9 at 37°C							
0	130	4.0	3.1	-	42.3	32.5	-
0.01	165	4.3	2.6	1	40.8	24.7	1
0.1	147	4.8	3.3	1	38.3	26.1	1
0.5	124	6.5	5.2	2	39.3	31.7	1
1.0	133	2.5	1.9	1	29.5	22.2	1
2.5	125	0	0	-	0.8	0.6	1
10 CP	70	23.8	34.0	11*	223.0	318.6	10*

$$\text{Ratio}^{(1)} = \frac{\text{Mean number of revertants per } 10^6 \text{ survivors per treated plate}}{\text{Mean number of revertants per } 10^6 \text{ survivors per control plate}}$$

* Reproducible values of greater than twice the control value are considered to indicate a mutagenic response.

Table 1.2b - Mitotic gene conversion in liquid cultures of Saccharomyces cerevisiae JD1 after treatment with sodium isopropyl xanthate in water, 4-nitroquinoline-N-oxide (NQO) or cyclophosphamide (CP) in the presence and absence of rat liver S9 fraction - raw data

mg/ml	Revertants per plate								Survivors x 10^4 (per plate)		
	HISTIDINE LOCUS				TRYPTOPHAN LOCUS						
Expt 1A 1 hr -S9 at room temperature											
0	5	0	5	3	24	11	C	C	239	214	165
0.01	1	1	1	2	9	5	3	9	149	85	E
0.1	3	8	4	1	17	34	8	C	156	173	202
0.5	1	1	1	3	11	10	8	13	210	153	E
1.0	2	2	1	0	8	18	15	22	154	185	164
5.0	0	0	0	0	0	14	7	4	186	209	133
0.001 NQO	48	65	78	52	310	286	172	269	6	8	1
Expt 1B 1 hr +S9 at 37°C											
0	1	1	3	5	8	8	4	8	161	143	132
0.01	3	1	2	0	8	4	4	7	96	145	127
0.1	1	3	1	0	7	2	5	7	124	112	152
0.5	0	1	1	3	4	2	2	8	170	85	135
1.0	0	0	0	1	7	5	5	2	141	208	212
5.0	0	0	0	0	2	0	0	0	103	82	125
10 CP	1	4	3	2	6	13	11	15	150	100	107
Expt 1C 4 hr +S9 at 37°C											
0	1	3	4	3	24	34	13	7	158	106	80
0.01	1	1	2	1	7	C	6	10	161	133	184
0.1	1	1	0	1	4	2	9	5	126	125	130
0.5	4	2	3	1	17	16	12	11	161	121	77
1.0	0	0	0	2	6	8	6	11	58	108	110
5.0	0	0	0	0	0	0	0	0	31	82	65
10 CP	21	C	23	C	197	224	162	173	131	67	121

Table 1.2b - Contd

Expt 2A -S9 for 1 hr at room temperature											
0	3	8	5	11	49	39	34	43	154	138	169
0.01	5	7	4	7	54	72	42	51	187	135	122
0.1	7	9	7	10	46	44	48	50	133	165	144
0.5	4	2	2	1	38	53	37	39	130	107	68
1.0	0	0	0	0	32	40	37	34	129	108	152
2.5	0	0	0	0	0	0	0	0	112	67	67
0.0001 NQO	17	13	20	13	95	77	118	101	115	91	96
Expt 2B +S9 after 1 hr at 37°C											
0	5	2	5	C	56	48	39	51	109	142	85
0.01	4	8	4	3	41	42	46	29	144	112	61
0.1	6	5	4	6	31	38	48	41	177	152	129
0.5	4	2	4	3	31	38	36	45	155	136	134
1.0	1	0	1	1	29	36	23	32	122	128	105
2.5	0	0	0	0	1	1	0	0	113	109	146
10 CP	9	14	5	4	46	43	39	45	150	106	145
Expt 2C +S9 after 4 hrs at 37°C											
0	4	4	1	7	43	50	34	42	140	114	135
0.01	5	3	4	5	45	39	46	33	184	156	156
0.1	4	5	2	8	42	34	31	46	170	121	150
0.5	7	5	6	8	40	43	44	30	119	121	131
1.0	0	2	2	6	34	28	30	26	120	137	142
2.5	0	0	0	0	0	1	1	1	136	78	161
10 CP	23	23	24	25	214	227	221	230	78	61	72

C = contaminated

E = plate lost due to experimental error

APPENDIX II

CYTOGENETICS REPORT

Title: Toxicity studies with Mining Chemicals: In vitro chromosome studies with sodium isopropyl xanthate (SIX).

Responsible Practitioners: G. Hodson-Walker.

Work done: The cytogenetic effects of SIX was investigated in monolayer slide cultures of rat liver (RL_4) cells.

RESULTS

Initially cultures of RL_4 cells were exposed to 1.0, 2.0 or 4.0 µg/ml of SIX. The only finding of note was a substantial increase in the frequency of chromatid gaps and a single cell containing 3 exchange figures in cultures exposed to 1.0 µg/ml (Tables 2.1a and 2.1b). Cultures exposed to 2.0 or 4.0 µg/ml showed no significant increase in the incidence of chromosome damage.

A second experiment was then carried out in which cultures of RL_4 cells were exposed to 0.25, 0.5 or 1.0 µg/ml of SIX. In this study there was no significant increase in the incidence of chromosome damage in any of the cultures exposed to SIX (Tables 2.2a and 2.2b), but due to a low yield of metaphases (i.e. <300 per dose level) a third assay was carried out.

In the third study of identical design to the first, the frequency of chromosome damage did not differ significantly from the control values (Tables 2.3a and 2.3b).

In all three studies cultures exposed to the positive control substance, DMBA, showed a marked increase in chromosome damage.

G. Hodson-Walker

G. Hodson-Walker
Responsible Practioner
Date: 1.5.81.

Table 2.1a - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA)

Compound	Conc. µg/ml	No. of cultures	No. of cells analysed	% cells showing				Frequency per cell of					
				Polyploidy (1)	Chromatid gaps (2)*	Multiple chromatid damage (3)	Chromatid aberrations (4)*	Chromosome aberrations (5)	Chromatid gaps *	Chromatid breaks (6)*	Chromatid exchanges	Chromosome breaks	Chromosome exchanges (7)
Sodium isopropyl xanthate	0	3	254	1.6	2.0	0	0	0	0.020	0	0	0	0
	1	3	300	2.0	12.7	0	0.7	1.0	0.167	0.003	0.010	0.017	0
	2	3	300	1.3	2.3	0	0	0.3	0.023	0	0	0.003	0
	4	3	149	0.7	0.7	0	0.7	0	0.007	0.007	0	0	0
DMBA	1	2	132	2.3	28.0	5.3	5.3	0.8	0	0	0.14	0.008	0

* Cells with multiple chromatid damage excluded
(1) Polyploidy + endoreduplication (2) Gaps + iso-gaps (3) Gaps + breaks exchanges or any combination
(4) Breaks + single fragments + exchange figures (5) Acentric fragments + dicentrics + rings + translocations
(6) Single fragments + chromatid breaks (7) Dicentrics + translocations + rings.

Table 2.1b - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-diemthylbenzanthracene (DMBA) [Raw data]

Compound	Conc. µg/ml	CYT/ 307	No. of cells analysed	Number of aberrations per culture										
				Poly-ploidy	Endo-redup-lication	Chromatid gaps	Iso-gaps	Chromatid breaks	Single fragments	Acentric fragments	Exchange figures	MCB	MCA	Dicen-trics
Sodium isopropyl xanthate	0	007	100	2		3								
	0	010	100	2		1								
	0	013	54			1								
	1	005	100	3		18			1					
	1	009	100		1	16	1			2				
	1	014	100	2		15				3	3			
	2	002	100	2		5								
	2	008	100			1				1				
	2	011	100	2			1							
	4	001	3											
	4	013	46	1		1			1					
	4	012	100											
DMBA	1	004	32	1		11					3			
	1	006	100	2		77	3		1	1	16			

CYT = cytogenetic code number
MCB = multiple chromatid breaks
MCA = multiple chromatid aberrations

Table 2.2a - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA)

Compound	Conc. µg/ml	No. of cultures	No. of cells analysed	% cells showing					Frequency per cell of				
				Polyploidy (1)	Chromatid gaps (2)*	Multiple chromatid damage (3)	Chromatid aberrations (4)*	Chromosome aberrations (5)	Chromatid gaps *	Chromatid breaks (6)*	Chromatid exchanges	Chromosome breaks	Chromosome exchanges (7)
Sodium isopropyl xanthate	0	3	234	0.4	3.8	0	1.7	0.4	0.047	0.004	0.017	0.004	0
	0.25	3	273	0	0	0	0	0	0	0	0	0	0
	0.5	3	272	0.7	1.8	0	0.4	0.4	0.026	0.004	0	0.004	0
	1.0	3	108	0.9	4.6	0	0	1.9	0.074	0	0	0.019	0
DMBA	1	2	114	0	12.3	1.8	4.4	0	0.184	0.018	0.035	0	0

* Cells with multiple chromatid damage excluded
(1) Polyploidy + Endureduplication (2) Gaps + iso-gaps (3) Gaps + breaks + exchanges or any combination
(4) Breaks + single fragments + exchange figures (5) Acentric fragments + dicentrics + rings + translocations
(6) Single fragments + chromatid breaks (7) Dicentrics + translocations + rings

Table 2.2b - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA) [Raw data]

Compound	Conc. µg/ml	CYT/ 348	No. of cells analysed	Number of aberrations per culture										
				Poly-ploidy	Endo-redup-lication	Chromatid gaps	Iso-gaps	Chromatid breaks	Single fragments	Acentric fragments	Exchange figures	MCB	MCA	Dicen-trics
Sodium isopropyl xanthate	0	004	100			11		1		1	4			
	0	008	100	1										
	0	014	34											
	0.25	001	100											
	0.25	006	100											
	0.25	007	73											
	0.5	002	72							1				
	0.5	003	100	1										
	0.5	012	100	1		6								
	1.0	009	100	1		1	1	1		2				
	1.0	010	6											
	1.0	013	2			7								
DMBA	1	005	72			1		1	1		4			
	1	011	42			20		1					1	

CYT = cytogenetic code number
MCB = multiple chromatid breaks
MCA = multiple chromatid aberrations

Table 2.3a - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA)

Compound	Conc. µg/ml	No. of cultures	No. of cells analysed	% cells showing					Frequency per cell of				
				Polyploidy (1)	Chromatid gaps (2)*	Multiple chromatid damage (3)	Chromatid aberrations (4)*	Chromosome aberrations (5)	Chromatid gaps *	Chromatid breaks (6)*	Chromatid exchanges	Chromosome breaks	Chromosome exchanges (7)
Sodium isopropyl xanthate	0	3	300	1.7	0.3	0	0	0	0.003	0	0	0	0
	1	3	300	0	0	0	0	0	0	0	0	0	0
	2	3	300	1.3	0.3	0	0.7	0	0.003	0.003	0.003	0	0
	4	3	279	1.1	0.7	0	0	0	0.007	0	0	0	0
DMBA	1	2	70	0	5.7	0	7.1	0	0.057	0	0.086	0	0

* Cells with multiple chromatid damage excluded
(1) Polyploidy Endoreduplication (2) Gaps + iso-gaps (3) Gaps + breaks + exchanges or any combination
(4) Breaks + single fragments + exchange figures (5) Acentric fragments + dicentrics + rings + translocations
(6) Single fragments + chromatid breaks (7) Dicentrics + translocations + rings.

Table 2.3b - Metaphase chromosome analysis of RL_4 cells after exposure to sodium isopropyl xanthate or 7,12-dimethylbenzanthracene (DMBA) [Raw data]

Compound	Conc. µg/ml	CYT/ 355	No. of cells analysed	Number of aberrations per culture										
				Poly-ploidy	Endo-redup-lication	Chromatid gaps	Iso-gaps	Chromatid breaks	Single fragments	Acentric fragments	Exchange figures	MCB	MCA	Dicen-trics
Sodium isopropyl xanthate	0	004	100	3										
	0	009	100	1		1								
	0	011	100	1										
	1	001	100											
	1	006	100											
	1	013	100											
	2	003	100			1			1		1			
	2	008	100	1										
	2	014	100	3										
	4	005	100	2		2								
	4	007	100											
	4	012	79	1										
DMBA	1	002	43			2					4			
	1	010	27			2					2			

CYT = cytogenetic code number
MCB = multiple chromatid breaks
MCA = multiple chromatid aberrations

APPENDIX 3

Compound Control and Formulation Chemistry Report

Title of Main Report:	Toxicity studies with Mining Chemicals: Short-term mutagenicity studies with sodium isopropyl xanthate.
Author:	[redacted]
Summary:	Data concerning test and control substances and their formulations are reported.

Test substance

Data describing the test substance used in this study are tabulated below:

Name	:	Sodium isopropyl xanthate
Source	:	Shell Santiago, Chile
CAS Ref. No.	:	[140-93-2]
Code No.	:	SF 113
Batch No.	:	SECADO 1734
Appearance	:	Yellowish pellets
Purity	:	Approximately 80-90% by mass
Date released	:	22nd June 1979, 17th June 1980 and 20th November 1980

The identity of the test substance was proved by infra-red spectrometry. The same technique was used to demonstrate the stability before re-release.

Formulation of test substance

The test substance was formulated as solutions in sterile distilled water. The concentrations ranged from 400 mg/ml to 0.001 mg/ml. These were made by dissolving a known mass of test substance in sterile distilled water and diluting as required.

Stability of formulations of test substance

Sodium isopropyl xanthate is produced by the interaction of isopropanol and carbon disulphide in aqueous sodium hydroxide. Xanthates are hydrolysed in aqueous solution, but this is not a rapid reaction in water at ambient temperature. The major use of alkali metal xanthates is as collectors in the flotation of metallic sulphide ores, which is done in aqueous solution.

On the basis of an examination of the chemistry and usages of xanthates, it was judged that aqueous solutions of sodium isopropyl xanthate would be stable for one working day.

Control substances

The control substances available for use in this study are shown below:

Control Substances	Source	Batch No.	Vehicle	Concentration
Benzo(a)pyrene Benzo(a)pyrene	Koch-Light Koch-Light	62991 76096	Dimethyl Sulphoxide (DMSO)	0.25, 0.5 and 1 mg/ml
Cyclophosphamide	Koch-Light	74841	Water	25 mg/ml
Neutral Red	G. T. Gurr	-	Water	1 mg/ml
4-Nitro-quinoline-N-oxide	ICI Ltd. Central Toxicology Laboratory	3757-10	DMSO	0.01, 0.1 and 1 mg/ml
Sodium azide	Fisons	40	Water	1 mg/ml
7,12-dimethyl-benzanthracene	Eastman Kodak	A6B	DMSO	0.5 mg/ml

Data on which shelf lives of formulations of control substances were based are shown below. Stability studies were not necessarily carried out on the same batches as were used for this study, but are considered to be independent of the batch.

Formulation and stability of benz(a)pyrene

Benz(a)pyrene (also termed 1,2-benzopyrene or 3,4-benzopyrene) Fig. 1 is normally formulated either as a solution in acetone or in dimethyl sulphoxide (DMSO). The stability of benz(a)pyrene as a solution in acetone or DMSO has been determined. This was done by analysis of fresh and stored solutions of benz(a)pyrene by high performance liquid chromatography using the following analytical conditions:-

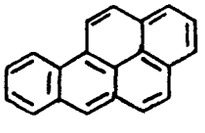
Column	-	0.25 m x ¼" O.D., 4 mm I.D. stainless steel
Packing	-	10 micron Spherisorb ODS 18
Detector	-	Cecil CE 212 Ultraviolet detector operating at 365 nm.
Solvent	-	80/20 (v/v) acetonitrile/distilled water (degassed)
Flow rate	-	1.6 ml/min.

The concentration of solutions used for stability studies and conditions of storage are summarised in the table.

Solvent	Concentration of Benz(a)pyrene (µg/ml)	Storage Conditions
DMSO	1000	Clear glass flask in the light @ ≃ +20°C
Acetone	50	Clear glass flask in the dark @ ≃ -18°C
Acetone	37.5	Clear glass flask in the dark @ ≃ -18°C
Acetone	12.5	Clear glass flask in the dark @ ≃ -18°C

The chromatograms obtained from fresh solutions were identical with those obtained from solutions stored for four weeks. This demonstrates that there is no appreciable decomposition when solutions of Benz(a)pyrene are stored as shown in the table. This is substantiated by results from in vitro mutagenicity testing, when solutions up to four weeks old retain their activity.

Fig. 1



Benz(a)pyrene,
1,2-benzopyrene
3,4-benzopyrene

Formulation and stability of cyclophosphamide

Cyclophosphamide is formulated as an aqueous solution. Such solutions decompose on storage. According to the British Pharmaceutical Codex, aqueous solutions of cyclophosphamide may be kept for a few hours at room temperature. All solutions of cyclophosphamide expire on the day of formulation.

Formulation and stability of neutral red

Neutral Red is formulated as an aqueous solution. The stability of an aqueous solution (1 mg/ml) of neutral red stored at room temperature (≈ 20°C) in the dark in a volumetric flask for 34 days has been determined. This was done by comparing a freshly prepared solution with a solution stored for 34 days, as above by a spectrophotometric technique.

The method used was as follows:-

0.5 ml of the fresh and stored solution were diluted to 250 ml with distilled water. The resulting dilute solutions were examined by spectrophotometry at wavelengths between 450 and 800 nm using water in the reference beam.

Both fresh and stored solutions of the neutral red gave similar spectra and were considered to be stable over this period.

This study demonstrated that a shelf life of 4 weeks can be assigned to aqueous solutions of neutral red.

This is substantiated by results from in vitro mutagenicity testing, when solutions up to 4 weeks old retain their activity.

Formulation and stability of 4-nitroquinoline-N-oxide

4-Nitroquinoline-N-oxide (NQO) is normally formulated as a solution in dimethyl sulphoxide (DMSO). The stability of NQO as a solution in DMSO has been determined. This was done by analysing fresh and stored solutions of NQO in DMSO (1 mg/ml) by high performance liquid chromatography using the following analytical conditions.

Column	-	0.25 x ¼" O.D., 4 mm I.D. stainless steel
Packing	-	10 micron Spherisorb ODS 18
Detector	-	Cecil CE 212 Ultraviolet detector operating at 365 nm.
Solvent	-	80/20 (v/v) acetonitrile/distilled water (degassed)
Flow rate	-	1.6 ml/min.

A solution of NQO in DMSO (1 mg/ml) was stored at room temperature (≃ 20°C) in a clear glass stoppered container for four weeks. A fresh solution was prepared, and both were analysed as described. The chromatograms obtained from the fresh and stored solutions were identical. This demonstrates that there is no appreciable decomposition when solutions of NQO in DMSO are stored as described. This is substantiated by results from in vitro mutagenicity testing, when solutions up to four weeks old retain their activity.

Formulation and stability of sodium azide

Sodium azide is formulated as an aqueous solution. The stability of an aqueous solution (0.25 mg/ml) of sodium azide stored in a dark glass bottle at room temperature (≃ 20°C) for 37 days has been determined. This was done by comparing a freshly prepared solution with a solution stored for 37 days, as above, by a colorimetric technique.

The method used was as follows:- A portion of the stored solution (1.0 ml) was mixed with a 0.5% aqueous solution of ferric sulphate (1.0 ml). The resulting red colour was measured spectrophotometrically using a 5 mm path length and a 2.5 mg/ml aqueous solution of ferric sulphate in the reference beam. A fresh solution of sodium azide in water (0.25 mg/ml) was analysed similarly.

Both fresh and stored solutions of sodium azide gave similar spectra (λ max 452 nm, 0.593 Absorbance for fresh; λ max 454 nm, 0.585 Absorbance for stored solutions). The sodium azide solution had therefore retained its strength during the 37 days' of storage.

This study demonstrated that a shelf life of 4 weeks can be assigned for aqueous solutions of sodium azide.

This is substantiated by results from in vitro mutagenicity testing, when solutions up to 4 weeks retain their activity.

Formulation and stability of 9,10-dimethyl-1,2-benzanthracene

9,10-Dimethyl-1,2-benzanthracene (DMBA) is normally formulated as a solution in dimethyl sulphoxide (DMSO). Studies of the stabilities of solutions containing 10 mg/ml and 0.5 mg/ml of DMBA in DMSO have been carried out using high performance liquid chromatography. The following analytical conditions were employed:-

Column	-	0.25 m x ¼" O.D., 4 mm I.D. stainless steel
Packing	-	10 micron Spherisorb ODS 18.
Detector	-	Cecil CE 212 Ultraviolet detector operating at 365 nm
Solvent	-	80/20 (v/v) acetonitrile/distilled water (degassed)
Flow rate	-	1.6 ml/min.

Solutions of DMBA in DMSO (10 and 0.5 mg/ml) were stored in stoppered clear glass vessels at room temperature (≃ 20°C) for up to five weeks. Fresh solutions were prepared and the old and fresh solutions were analysed as described. The chromatograms obtained from old and fresh solutions were identical, indicating that no decomposition had taken place.

When stored solutions of DMBA in DMSO were used as positive controls for <u>in vitro</u> mutagenicity testing, it was found that there had been a decrease in biological activity. There was thus a conflict between the results of chemical and biological analysis. The reason for this conflict was not resolved, but solutions of DMBA are not used beyond the date of formulation.

[redacted]

[redacted], B.A., Ph.D., M.R.S.C., C. Chem.
Responsible Practitioner
Date. 1st May 1981

QUALITY ASSURANCE STATEMENT

REPORT NUMBER: TLGR.80.156

EXPERIMENT NUMBER: IMX-1628

REPORT TITLE: Toxicity studies with Mining Chemicals: *In vitro* genotoxicity studies with sodium isopropyl xanthate.

STUDY DIRECTOR: T.M. Brooks

The procedures that were used in this study have been inspected and this report has been audited to ensure that it accurately describes the methods used and that the reported results accurately reflect the raw data of the study.

Date of inspection or audit.	Principal Subject	Date of written QA report to management
25 to 29. 2.80	Microbial assay	4. 3.80
14 to 16. 5.80	Chromosome assay	4. 6.80
29. 4.80	Chemical formulation	7. 5.80
22. 4.81	Study report	22. 4.81

[redacted]

QUALITY ASSURANCE INSPECTOR.

TLGR.80.156

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Page 1/35

GROUP RESEARCH REPORT

TLGR.0047.78

TOXICOLOGY OF MINING CHEMICALS: ACUTE TOXICITY, SKIN AND EYE IRRITANCY AND SKIN SENSITIZATION POTENTIAL OF SODIUM ISOPROPYL XANTHATE

SICC/CIMS Budget Ref. 50070612

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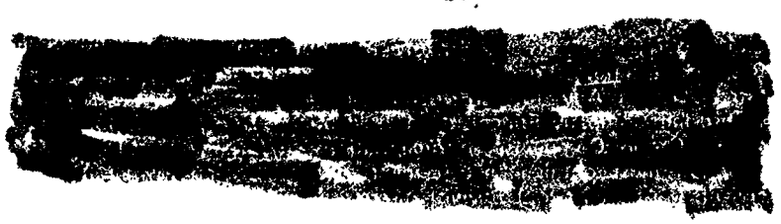

SHELL TOXICOLOGY LABORATORY (TUNSTALL)

Group Research Report TLGR.0047.78

Title: Toxicology of Mining Chemicals: Acute toxicity, skin and eye irritancy and skin sensitizing potential of Sodium Isopropyl Xanthate.

Authors: [redacted]

Reviewed by: [redacted]

Work done by: Experimental Toxicology Division of Shell Toxicology Laboratory (Tunstall).

Object: To determine the acute oral and percutaneous toxicity, skin and eye irritation and skin sensitizing potential of the test material.

Summary:

1. The acute oral LD_{50} of the test material in rats was approximately 800 mg/kg.

2. The acute (24 h) percutaneous LD_{50} of the test material in rats was greater than 1000 mg/kg.

3. A single 24 h application of the test material to occluded rabbit skin was slightly irritating.

4. The test material was severely irritating to rabbit eyes.

5. The test material was a moderate skin sensitizer in guinea-pigs.

[redacted]

[redacted], B.V.M.S.,D.V.M.,Ph.D.,M.R.C.V.S.,M.R.C.Path.
Director, Shell Toxicology Laboratory (Tunstall).

Date: March, 1978.

INTRODUCTION

Sodium isopropyl xanthate is destined for use as a collector (promoter) in froth flotation concentration of sulphide ores of copper [redacted] industry.

The toxicological [redacted] here was undertaken to determine the acute handling hazards associated with use of the chemical.

[redacted]

[redacted]

MATERIALS AND METHODS

Sample

A sample of the chemical was received from Shell-Flot, Chile (reference No. SF 113).

Animals

Species	Strain/Breed	Source
Rat	Wistar	Shell Toxicology Laboratory (Tunstall), Breeding Unit.
Guinea-pig	'P' Strain	Shell Toxicology Laboratory (Tunstall), Breeding Unit.
Rabbit	New Zealand White	Ranch Rabbit, Crawley, Sussex.

Acute oral toxicity

Male and female rats aged approximately 12 weeks, were used at each dose level. Four animals of one sex were housed in each cage. The animals were weighed, fasted overnight and the calculated dose of material administered by intraoesophageal intubation using a ball point needle fitted to a syringe. After dosing food and water were freely available throughout a 9 day observation period.

Acute percutaneous toxicity

The method of Noakes and Sanderson (Appendix I) was used to determine the acute (24 h) LD_{50} in groups of male and female rats. Observation was continued for 9 days.

Primary skin irritation

The occlusive patch test of Draize (Appendix II) was used to assess the skin irritation to intact and abraded rabbit skin.

Eye irritation

The method of Draize as described in the Federal Register (Appendix III) was used to assess the eye irritation in groups of three rabbits.

Skin sensitization

The skin sensitizing potential of the material was assessed using groups of 10 male and female guinea-pigs in the Magnusson and Kligman maximization test (Appendix IV) following preliminary range finding tests to determine suitable concentrations for intradermal induction and topical induction and challenge.

RESULTS

Acute oral toxicity

Dosing groups of 4 male and 4 female rats with a 50% w/v solution in water resulted in the following mortalities:

Dose (mg/kg)	Daily mortality (days 1-9)								Cumulative mortality (9 days)		
	Day 1		Day 2		Day 3		Day 9				
	M	F	M	F	M	F	M	F	M	F	Total
200			1						1/4	0/4	1/8
400									0/4	0/4	0/8
800		1	2	1		1			2/4	3/4	5/8
1600	4	3		1					4/4	4/4	8/8

Using these figures the acute oral LD_{50} was estimated to be approximately 800 mg/kg.

Rats showed signs of lethargy and died following a period of coma.

Acute percutaneous toxicity

The application of a 50% w/v solution in water for 24 hours to groups of 4 male and 4 female rats resulted in the following mortalities:

Dose (mg/kg)	Daily mortality (days 1-9)								Cumulative mortality (9 days)		
	Day 1		Day 2		Day 3		Day 9				
	M	F	M	F	M	F	M	F	M	F	Total
1000				1					0/4	1/4	1/8

On the basis of these figures the acute percutaneous LD_{50} was estimated to be greater than 1000 mg/kg - the maximum that could be applied to the skin.

Rats showed no signs of toxic reaction.

Primary skin irritation

The erythema and oedema resulting from the application of powdered chemical to rabbit skin were scored at 24 hours, 72 hours and 7 days using standard scales ranging from 0 to 4. The results are tabulated below:

Rabbit number and sex	Response											
	Abraded skin						Non abraded skin					
	24 hours		72 hours		7 days		24 hours		72 hours		7 days	
	E	O	E	O	E	O	E	O	E	O	E	O
1 M	1	0	0-1	0	0	0	0-1	0	0-1	0	0	0
2 M	0-1	0	0	0	0	0	0-1	0	0	0	0	0
3 M	1	0	0-1	0	0	0	1	0	0-1	0	0	0
Mean	0.8	0	0.3	0	0	0	0.7	0	0.3	0	0	0

Using these figures the material would be classified as slightly irritating to rabbit skin.

Eye irritation

The conjunctival redness chemosis and discharge, corneal opacity and damage to the iris following the instillation of 100-200 mg of powder into the conjunctival sac of three rabbit eyes was scored using standard scales. The results are tabulated below:

	Mean response				
	1-2 hours	1 day	2 days	3 days	7 days
Conjunctiva					
Redness	2	2	2	1.7	1.3
Chemosis	1	1	1	0.7	0
Discharge	1	0	0.3	0	0
Cornea					
Opacity	2	2	2	2	2
Area	4	4	4	4	4
Iris	2	2	2	2	1.3

On the basis of these scores the material would be classified as severely irritating to rabbit eyes.

Immediately on instillation into the rabbit eyes a moderate pain reaction was observed.

Skin sensitization

Following initial range finding tests the following concentrations were used in the method of Magnusson and Kligman.

Intradermal induction: 1% w/v in H_2O
Topical induction: 10% w/v in H_2O
Topical challenge: 5% w/v in H_2O

The erythema resulting from the topical challenge was scored on a four point scale (- ve; trace; + ve; ++ ve) immediately on removal of the challenge patch and 24 and 48 hours later. The results are tabulated below:

Animal number	Response					
	Immediate		24 hours		48 hours	
	Male	Female	Male	Female	Male	Female
Test						
1	-	tr	+	+	+	+
2	tr	tr	+	tr	tr	-
3	-	tr	-	+	-	tr
4	tr	tr	+	+	tr	tr
5	-	tr	-	-	-	-
6	tr	+	+	+	+	+
7	tr	-	tr	tr	-	-
8	-	+	-	+	-	tr
9	+	+	+	+	tr	tr
10	-	-	tr	-	-	-
Control						
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-

Based on the number of animals showing a response, the degree of intensity of the response and its persistence, the material is considered to be a moderate skin sensitizer in guinea-pigs.

Archives

The data on which this report is based is filed under Experiment number 1385.

S. L. Cassidy.

S. L. Cassidy, B.Sc.

D. G. Clark, B.Sc., Ph.D., M.I.Biol.

APPENDIX I

ACUTE PERCUTANEOUS TOXICITY

The method of Noakes and Sanderson* was used.

Groups of rats of each sex, aged 12-13 weeks, were used at each dose level. The test material was placed onto the shorn dorso-lumbar skin and bandaged into contact with the skin using an impermeable dressing of aluminium foil and water proof plaster. The rats were housed individually over the 24 hours exposure period during which time they were deprived of food but allowed water ad libitum.

After 24 hours the dressings were removed and the exposed area was washed with a tepid dilute detergent solution. The rats were then housed in cages of four of one sex and observed for signs of toxicity during the following 9 days.

*Noakes, D. N. and Sanderson, D. M., (1969). A method for determining toxicity of pesticides. Br. J. industr. Med., 26, 59-64.

APPENDIX II

PRIMARY IRRITATION OF THE SKIN

The method of Draize* was used.

Primary irritation of the abraded and intact skin of each of three male rabbits was measured. The dorsal hair between the shoulders, and the hindquarters was closely shorn by means of electric clippers. A 2 x 2 cm area of the shorn skin was abraded using a fine hypodermic needle, the injuries being deep enough to disturb the stratum corneum but not sufficiently deep to cause bleeding. Lint patches (2 x 2 cm) were applied to the abraded and intact skin and 0.5 ml test material was applied to each. The patches were covered by an occlusive polyethylene film which was secured in position by means of an elastic adhesive bandage (3" Poroplast). The patches were left in place for 24 hour.

Following removal of the patches the skin reactions were assessed visually for the degree of erythema and oedema using the 0-4 scale tabulated below at 24 and 72 hours. Seven days after the application of the test material a final visual assessment was made.

No erythema	=	0	No oedema	=	0
Pale pink	=	1	Soft skin	=	1
Redness	=	2	Oedema	=	2
Severe redness	=	3	More definite oedema	=	3
Beet redness	=	4	Severe oedema	=	4

*Draize, J. H., (1969).
'Dermal Toxicity' in "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.
Association of Food and Drug Officials of the United States of America.

APPENDIX III

EYE IRRITATION

The method of Draize as described in the U.S. Federal Register* was used.

The test compound was instilled into the conjunctival sac of one eye of each of three rabbits; the untreated eyes served as controls. A visual assessment of irritancy was made 1 to 2 hours after instillation and again at 1, 2, 3 and 7 days, thence every 4 days until eye irritancy was no longer observed using the standard scales detailed below:

CORNEA

No ulceration or opacity . . . 0

Scattered or diffuse areas of opacity (other than slight dulling or normal lustre), details of iris clearly visible 1

Easily discernible translucent areas, details of iris slightly obscured 2

Nacreous areas, no details of iris visible, size of pupil barely discernible 3

Complete corneal opacity, iris not discernible 4

IRIS

Normal 0

Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination thereof), iris still reacting to light (sluggish reaction is positive) 1

No reaction to light, haemorrhage, gross destruction (any or all of these) 2

CONJUNCTIVAE

Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal 0

Some vessels definitely injected . . 1

Diffuse, crimson red, individual vessels not easily discernible . . . 2

Diffuse beefy red 3

CHEMOSIS

No swelling 0

Any swelling above normal (includes nictitating membrane) 1

Obvious swelling with partial eversion of lids 2

Swelling with lids about half closed 3

Swelling with lids more than half closed 4

DISCHARGE

No discharge 0

Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1

Discharge with moistening of lids and hairs just adjacent to lids . . 2

Discharge with moistening of lids and hairs and considerable area around the eye 3

*Federal Register, 28 (110), 6.6.1963. para. 191.12 Test for eye irritants.

APPENDIX IV

SKIN SENSITIZATION

The guinea-pig maximization procedure of Magnusson and Kligman* was used to assess the skin sensitizing potential of the test material.

A preliminary screen was carried out using groups of two male and two female guinea-pigs to determine the concentrations of test material to be used for intradermal induction, topical induction and topical challenge.

In the test itself groups of ten male and ten female guinea-pigs were used with a further five males and five females as controls.

Induction

Induction was accomplished in two stages:

(i) Intradermal injection

Two rows of three injections were made: one on each side of the midline in the shorn skin of the shoulder region as follows:

Test animals	Controls
2 x 0.1 ml Freund's complete adjuvant	FCA
2 x 0.1 ml Test material in solvent	Solvent
2 x 0.1 ml Test material in 50:50 FCA/solvent	50:50 FCA/solvent

The injection sites were just within the boundary of a 4 x 4 cm shaved area.

(ii) Topical application

One week after the intradermal injections the same area was clipped free from hair. A 4 x 4 cm patch of Whatman No. 3 mm filter paper was soaked in a solution of the test material, placed over the injection sites of the experimental animals and covered by overlapping plastic adhesive tape (1½" Blenderm). This in turn was firmly secured by an elastic adhesive bandage (3" Poroplast). The dressing was left in place for 48 hours.

APPENDIX IV (continued)

Challenge

The challenge procedure was carried out 2 weeks after topical induction. Challenge was accomplished by topical application of the challenge solution of the test material to the flank of both test and control groups of animals.

Hair was removed from a 3 x 3 cm area on the flank by clipping and then shaving with an electric razor. A 2½ x 2½ cm patch of Whatman No. 3 mm filter paper was soaked in the challenge solution and placed over the shaved area. This was then covered by overlapping adhesive tape (1½" Blenderm) which was in turn firmly secured by an elastic adhesive bandage (3" Poroplast). The patch was left in place for 24 hours and examination of the challenge site was immediately, 24 and 48 hours after removal of the dressing. Three hours before the 24 hour reading the treated skin was closely shaved by means of an electric razor.

*Magnusson, B. and Kligman, A. M., (1969).
The identification of contact allergens by animal assay.
The guinea-pig maximization test.
J. Invest. Derm., 52, 268-276.

TLGR.0047.78

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Date 15th February 1983

H. S. & E. REPORTS
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Dear Sirs,

Cyclopentadiene

We refer to our letter dated 10th February, 1983 relating to the 90 day study report on dicyclopentadiene and subsequent telephone conversation with your Dr. Thomas.

We attach a copy of the final report on Cyclopentadiene covering

1) a six hour LC_{50} vapor inhalation study in mice.

and

2) a nine-day vapor inhalation study in mice.

Yours [redacted]
For: SHELL INTERNATIONAL PETROLEUM COMPANY LIMITED

[redacted]

[redacted]

[redacted]

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FINAL REPORT

November 16, 1981

COMPOUND: Cyclopentadiene

SUBJECT: I. Six-Hour LC50 Vapor Inhalation Study on Mice
II. Nine-Day Vapor Inhalation Study on Mice

SPONSOR: EXXON Corporation
P. O. Box 45
Linden, New Jersey 07036

INITIATOR: [redacted]

[redacted]

Report 44-513
24 Pages
November 16, 1981

Cyclopentadiene
I. Six-Hour LC50 Vapor Inhalation Study on Mice
II. Nine-Day Vapor Inhalation Study on Mice

Authors: D. E. Dodd, L. C. Longo and W. M. Snellings

* * * * *

Abstract

Groups of six male and six female B6C3F1 mice were exposed by inhalation to 5465, 2762 or 1427 ppm of cyclopentadiene vapor in a single six-hour exposure. The LC50 (95% confidence limits), which is the concentration calculated to kill half of the animals, was 1778 (1064 to 2972) ppm for male mice and 3908 (3021 to 5055) ppm for female mice. Signs of respiratory distress were observed in the male mice at all three concentration levels.

A 9-day inhalation study in the same strain of mice followed the LC50 study. Groups consisting of 10 male and 10 female mice were exposed to concentrations of 2558,714, 244 or 0 ppm of cyclopentadiene vapor. Evaluation of toxic effects included determinations of body weight and food consumption, observations for behavioral and/or neuromuscular abnormalities, gross pathology, and organ weights. Respiratory distress (mouth breathing), decreased activity and coordination loss were observed in male and female mice of the 2558 and 714 ppm exposure levels. All mice exposed to these levels died prior to conclusion of the scheduled exposure period. Gross pathologic examination of these animals revealed no unusual postmortem findings. No deaths occurred in the animals exposed to 244 ppm. Only female mice of this level had a statistically significant increase in liver weight (both absolute and as a percentage of body weight). No gross hepatic lesions were noted at necropsy for this level; therefore, the biological importance of this finding is unclear. Comparisons between control and the 244 ppm exposure groups (male and female) showed no statistically significant differences for the other parameters examined in this study.

Objective

This study was designed to determine the 6-hour LC50 in male and female B6C3F1 mice exposed by inhalation to cyclopentadiene vapor. The results of the LC50 study were used to design a 9-day inhalation study, the purpose of which was to evaluate the toxic manifestations in mice exposed repeatedly to cyclopentadiene vapor.

Review of Literature

A literature review pertaining to the toxicologic effects of cyclopentadiene has been summarized by Dr. H. F. Smyth, Jr. and is attached (Appendix A). The TLV of 75 ppm has been determined solely on the standpoint of sensory irritation in humans.

Table of Contents

Materials and Methods

This study was conducted according to the specific protocol, CHF number 78-53-14300 and five amendments (dated July 24, 1979; July 27, 1979; September 17, 1979; October 5, 1979; and November 28, 1979) prepared for the 9-day cyclopentadiene study in rats and mice. Rat studies were deleted from this project (third amendment dated September 17, 1979) because preliminary studies indicated that the sensitivity to the toxic effects were so different that both species could not be exposed at the same levels. The rat study will be performed at a later time. Any exceptions to the protocol or amendments have been cited in this report.

A brief discription of the LC50 study is as follows: Groups of male and female B6C3F1 mice were exposed to different concentrations of cyclopentadiene vapor for six hours. Six mice per sex were randomly assigned to an exposure group. The inhalation chambers, the generation and analytical systems were the same as those in the 9-day study. Surviving mice were maintained and observed for toxic effects for at least 14 days following exposure. After this postexposure period, all surviving mice were necropsied. Unless otherwise indicated, all methods pertain to the 9-day study.

Test Material. Two 5-gallon drums of dicyclopentadiene (Lot CSTD BR#064-90-13) were received from EXXON Chemical Company, Baton Rouge, LA on August 20, 1979 and were assigned the sample numbers CHF 42-347A and CHF 42-347B. Pertinent chemical and physical properties, including compositional analysis, are presented in Tables I and II. The test material has been reported to be greater than 97% endo dicyclopentadiene and to be stable over a three-month period (Larrabee, 1979). Gaseous cyclopentadiene was generated by thermal degradation of liquid dicyclopentadiene. The efficiency of this thermal conversion was 99.9% (Larrabee, 1979). This high conversion percentage minimized the presence of dicyclopentadiene during cyclopentadiene vapor generation. General chemical and physical properties of cyclopentadiene are listed in Table III.

Animal Species and Source. Mice for the LC50 study (B6C3F1, 31 male and 32 female) were received from Charles River Laboratories, Wilmington MA on August 1, 1979. Mice for the 9-day study (B6C3F1, 62 male and 62 female) were received on August 30, 1979 from the same supplier. The mice for both studies were approximately 30 days old upon arrival and were housed in Room 146 and identified by a standard toe-clipping technique.

Upon arrival, mouse fecal samples were examined for intestinal parasites by zinc sulfate flotation. Visual examinations of the health and ophthalmologic status of all mice were performed prior to the initiation of exposures. Body weights were followed for one week prior to placement into exposure groups. Any animal judged to be unhealthy during the pre-exposure acclimation period was not assigned to an exposure group.

Animal Husbandry. Mice for both the LC50 and 9-day studies were separated by test groups and sex and were housed in stainless steel wire mesh cages. The animals were kept in Room 146 where temperature and relative humidity were

monitored daily. Water from the Municipal Authority of Westmoreland County (Greensburg, PA) was provided by both water bottles and an automatic watering system. Water and NIH-07 powdered feed (Ziegler Brothers, Inc., Gardners, PA) were available ad libitum except during inhalation exposure. A layer of Deotized Animal Cage Board® (the Upjohn Co., Kalamazoo, MI) was placed under each row of cages during non-exposure periods only. The photoperiod was 12 hours light and 12 hours dark.

Group Assignment and Size. Only animals with body weights within two standard deviations of the group mean for that sex on the day of group assignment were used in the study. Animals were assigned to test groups by employing a card-based random number system. Ten mice per sex per group were used in the 9-day study.

Inhalation Chamber Description. Chambers were located in Room 117. Each chamber had a volume of approximately 550 liters with the internal dimensions, in inches, of 45 x 29 x 26 and was constructed of tempered masonite with glass windows for animal observation. The internal walls of the chambers were sealed with sodium silicate. Chambers were operated at airflows of approximately 150 liters per minute. All chambers were maintained at a slight negative pressure relative to room atmosphere. Chamber temperature and relative humidity were recorded at least 3 times durin each exposure day.

Exposure Regimen and Study Schedule. The exposure dates for the LC50 study were August 14, 16, and 22, 1979 with the final sacrifice date of September 5, 1979 for all these studies. Target concentrations selected for the LC50 study were 5000, 2500, and 1250 ppm. Each single exposure period was for 6 hr.

The first exposure day of the 9-day study was September 10, 1979 and the final sacrifice day was September 22, 1979. Target concentrations of 2500, 750 and 250 ppm were selected for the 9-day study based upon the LC50 results. The mice were mock (air) exposed on the Thursday and Friday of the week prior to exposure initiation. Mice were exposed to cyclopentadiene vapor six hours per day for 5 consecutive days, allowed a 2 day rest and re-exposed for an additional 4 consecutive days. A two day staggered start schedule was followed in order to reduce the number of mice necropsied on a single day. All exposures began approximately 8 a.m. Control (air-exposed) mice were handled in an identical manner as cyclopentadiene-treated mice.

To compensate for any possible, but undetected, variation in chamber exposure conditions (i.e. concentration, temperature, relative humidity) the cages were rotated routinely within each chamber.

Vapor Generation. Cyclopentadiene vapor was generated by metering the liquid dicyclopentadiene into a pyrex tube furnace heated at approximately 450°C. Nitrogen was used as a carrier gas. The cyclopentadiene-N_2 mixture was cooled by passage through a circulating air condenser. Further dilution of the cyclopentadiene-N_2 mixture with air took place at the chamber intake plenum, where an airflow of approximately 150 liters per minute was maintained.

Analytical Method. A Perkin-Elmer 3920B gas chromatograph (GC) with a hydrogen flame ionization detector was used to analyze the chamber concentration of cyclopentadiene (conditions of operation are presented in Table IV). An automatic sampler was used to sample the atmosphere from the three test chambers, the air-control chamber and the exposure room. The electrical analog signal from the GC was integrated by a Spectra Physics Model 400 integrator data system. The integrator digital output was recorded on a magnetic cassette. The data were then transferred to a computer where the daily means for the 9-day study were calculated and stored in a computer file for future reference. Each test chamber was also analyzed periodically for le els of dicyclopentadiene vapor.

Daily Observations. On each exposure day, all animals were observed just prior to, during and immediately following exposure for any abnormalities in appearance or general behavior. Surviving mice of the LC50 study were observed for toxic effects during a 14-day postexposure period.

Modified Irwin Screen (9-day study only). The modified Irwin Screen (Irwin, 1966) was performed on five mice of each sex from all test levels (and control level) for signs indicative of behavioral and/or neuro-muscular abnormalities. Table V lists the parameters which were examined. Animals were observed on exposure days one, two, five, six, seven and prior to sacrifice. On each observation day, a new group of five mice were randomly selected from the remaining survivors.

Ophthalmologic Evaluation (9-day study only). The corneas of all mice were examined for gross lesions prior to the start of the inhalation study. Mice were culled if abnormalities were seen. Also, at necropsy the eyes of all mice were examined grossly.

Body Weight (9-day study only). The body weight of each mouse was determined in the morning preceding the first, second, fifth, sixth and seventh day of exposure, and again preceding sacrifice. This regimen deviated from the initial schedule stated in the protocol which was first, second, fifth, eighth, and preceding sacrifice. The weight recorded before the first exposure was considered the pre-exposure reference weight and was subtracted from each subsequent weight determination to obtain a change in body weight value.

Food Consumption (9-day study only). Food consumption (measured over a three-day period) for each mouse cage was determined weekly starting five days prior to the initiation of exposure. The amount consumed was divided by the number of mice in the cage then divided by the number of days (3) to determine the amount consumed per mouse per day. [Note: Food and water were removed from the cages during each exposure.]

Necropsy

I. LC50 Study. All animals which died during or survived the post-exposure observation period underwent necropsy.

II. Nine-Day Study. All survivors were necropsied on the day following the final exposure. Any moribund or dead animals found during the nine-day inhalation regimen were subjected to necropsy. Tissues were taken and fixed in neutral buffered 10% formalin from animals if treatment-related gross lesions were observed. After the ninth exposure day, all surviving mice were killed by severing the brachial blood vessels following anesthesia with methoxyflurane and were necropsied.

Organ Weights (9-day study only). The lungs, liver and kidneys of all animals, along with the testes of the males, were weighed at the time of sacrifice. Both absolute organ weights and organ weights expressed as percentage of total body weight for each exposure level were statistically compared to those of the control group.

Statistical Analysis. The LC50 and its 95% confidence limits were calculated by using a modification of the moving averages method of Thompson (1947). Results of the quantitative continuous variables (such as body weight changes) were intercompared among the test level groups and the control group by the following tests: Bartlett's homogeneity of variance (Sokal and Rohlf, 1969), analysis of variance (Snedecor and Cochran, 1967) and Duncan's Multiple Range (Duncan, 1955, 1957; Harter, 1960). The last test was used, when F from the analysis of variance was significantly high, in order to delineate which groups differed from control. When Bartlett's test indicated heterogeneous variances, the F-test (Sokal and Rohlf, 1969) was used to compare each group versus the control. When these individual F-tests were not significant, Student's t-test (Sokal and Rohlf, 1969) was used; if significant, the means were compared by the Cochran t-test (Snedecor and Cochran, 1967). The fiducial limit of 0.05 (two-tailed) was used as the critical level of significance.

Storage of Records. To the extent technically feasible and consistent with Good Laboratory Practices, Bushy Run Research Center will retain, safekeep and preserve all documents, data, and material relevant to the research program in the BRRC Archives.

RESULTS

I. LC50 Study

Chamber concentration. The mean (± standard deviation) chamber concentration of cyclopentadiene for each exposure group was 5465 ppm (± 405), 2762 ppm (± 86), and 1427 ppm (± 103). More than twenty atmospheric samples were taken from each chamber during exposure for concentration determination.

The dicyclopentadiene (DCPD) concentrations were monitored for each of the LC50 exposure chambers although for the first and second exposure days (2762 ppm and 5465 ppm) the attenuation of the gas chromatograph (GC) was too large to allow for evaluation of low concentrations of DCPD. On the last exposure day (1427 ppm) the GC attenuation was set at a lower level, and a concentration of approximately 2 to 3 ppm of DCPD was recorded throughout the exposure.

Observations and Gross Pathology

5465 ppm exposure -- male mice. During exposure, irregular respiration (labored breathing and gasping) was observed. Opaque corneas were seen in three mice following exposure and this condition persisted until the death of two of these mice. Immediately following exposure gasping continued, and three mice died (Table VI). At necropsy, gas-filled stomachs were found in these three mice. All remaining mice were dead by the end of the first postexposure day. Additional lesions were not observed in the remaining animals of this group.

5465 ppm exposure -- female mice. During exposure, mice appeared normal; however, all were dead by the end of the first postexposure day. At necropsy, one mouse had mottled lungs, but no other abnormalities were seen in any of the mice.

2762 ppm exposure -- male and female mice. Both sexes exhibited decreased locomotor activity towards the end of the exposure. Other activities appeared normal. Male mice that died (Table VI) displayed labored breathing and slight body trembling. At necropsy, these males had had mottled lungs and livers, as well as gas-filled stomachs and intestines. No female mice died, and no gross lesions were observed at necropsy.

1427 ppm exposure -- male and female mice. The male mice exhibited irregular respiration towards the end of the exposure. Following exposure the male mice appeared lethargic and the irregular respiration persisted. Two male mice died; however, female mice appeared normal during exposure and throughout the 14-day observation period. At necropsy, no significant gross abnormalities were found in any of the mice of this level.

LC50 Determination. Tabulation of the time of death, total number of mice dead for each exposure level and the LC50 values are shown in Table VI. The LC50 for male mice was 1778 ppm and for female mice, 3908 ppm.

II. Nine Day Study

Housing Conditions. The daily mean temperature and relative humidity for the holding room (146) and the exposure chambers can be found in Table VII. Room 146 was maintained within a temperature range of 22° to 23°C. The relative humidity ranged between 51 and 69%. For all groups, chamber temperature and relative humidity during the exposure did not exceed the range of 21° to 25°C and 39% to 54%, respectively, throughout the study period.

Chamber Concentration. Target concentrations for the high, intermediate, and low exposure levels were 2500, 750, and 250 ppm, respectively. Gas chromatographic analysis of chamber atmospheres resulted in mean measured concentrations of 2558, 714, and 244 ppm (Table VIII). Generation problems occurred in the high level on the fifth day of exposure, resulting in a low mean concentration (699 ppm) for this day. Since only one surviving animal was exposed on this day, the mean concentration for the

four previous days (2558 ppm) better represents the overall exposure concentration for the high level group. [Note: This remaining animal died after the fifth exposure.] Consequently, in this study 2558 ppm will be recorded as the exposure concentration for this group. The observed concentrations of dicyclopentadiene vapor in the test chambers are given in Table IX. Chamber atmosphere samples were analyzed approximately 5 to 7 times per exposure.

Appearance and Demeanor. Decreased locomotor activity, slight coordination loss, and abdominal breathing were observed in both males and females of the 2558 and 714 ppm exposure groups. Mice exposed to 240 ppm did not differ in appearance from control (0 ppm) mice.

Mortality. All mice from the 2558 and 714 ppm exposure groups died during the study period (Table X). No mortality was observed in the 240 ppm cyclopentadiene treated mice. [Note: One male mouse in the control (0 ppm) group died accidentally by getting its head caught in the cage.] The male mice appeared to be more sensitive to the effects of exposure than the females, as indicated by the earlier onset of mortality for the males.

Ophthalmologic Evaluation. The eyes of all mice appeared normal prior to the initiation of the study and at the terminal sacrifice period.

Modified Irwin Screen. Male and female mice exposed to either 2558 or 714 ppm exhibited the following irregularities prior to their death: abnormal righting reflex, abnormal pinch reflex of tail and/or toe decreased locomotor activity, tail elevatiom, abnormal gait, abdominal respiration, decreased response to provoking situations or stimuli, and slowed pupillary (females only) and corneal responses. Clonic and tonic convulsions were also observed in one mouse of the 714 ppm test group. Male and female mice exposed to 244 ppm cyclopentadiene and the male control mice appeared normal during the testing sessions. Two female control mice exhibited decreased activity during the testing, one mouse following exposure 6 and the other following exposure 7.

Body Weight. Mean body weight changes of mice from all exposure levels are presented in Tables XI (males) and XII (females). Males from the intermediate exposure group (714 ppm) had a significantly depressed body weight gain after one exposure day. All males from this group were dead by the next weighing interval. No significant differences were noted for the 244 ppm exposure group.

Similarly, before all female mice of the 714 ppm group had died, depression in body weight gain was observed. The body weight change values of the female mice in the 244 ppm group were similar to those of the control. The exception to this was a significantly higher body weight gain after nine completed exposures, but this is not considered a deleterious effect.

Food Consumption. No statistically significant differences between cyclopentadiene treated (244 ppm) and control mice were observed in males or females after four or nine completed exposures (Table XIII). Due to a high incidence of mortality at the 714 and 2558 ppm level, food consumption was not determined for these levels.

Necropsy. All mice of the two highest exposure levels died during the nine-day study. Observations noticed at necropsy of these mice were typical of post-mortem findings, e.g., dark red lungs, mottled livers, and fluid-filled intestines. Male and female mice from the 244 ppm and 0 ppm (control) exposure groups exhibited no gross abnormalities. No tissues were saved for histological examination.

Organ Weights. Organ weights for mice surviving the 9-day cyclopentadiene treatment are shown in Table XIV. The mean absolute liver weight and liver weight expressed as percentage of body weight of the female mice from the 244 ppm group were significantly ($P < 0.01$) higher than those of control mice. No other statistically significant differences were found between cyclopentadiene-treated and control groups.

DISCUSSIONS

Exposure to 2558 or 714 ppm of cyclopentadiene vapor in the 9-day repeated inhalation study resulted in respiratory difficulty and death for all exposed B6C3F1 mice. The male mouse appears more susceptible to the toxic effects of treatment than the female because the onset of mortality occurred much earlier in the male. This is supported by the considerably lower LC50 value for the male (1778 ppm vs. 3908 for the female). However, only in the female was there an apparent treatment-related effect noted in the lowest exposure level, 244 ppm, of the 9-day study. The only significant alteration noted in this level was an increase in liver weight. The biological significance of this is unclear since no gross hepatic lesions were noted at necropsy and no histopathology was conducted.

Reviewed and Approved by

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Table I
Chemical and Physical Properties of Dicyclopentadiene-97[1]

Chemical Name: EXXON Dicyclopentadiene-97

Chemical Formula: $C_{10}H_{12}$

CAS Registry Number: 77-73-6

Source: EXXON Chemical Company, Baton Rouge, LA

Batch or Lot Number: CSTD BR #064-90-13

Amount Acquired: Two 5-gallon drums

Physical Properties of Test Article:

Molecular Weight: 132.2

Specific Gravity (H_2O = 1): 0.9786 at 20/20°C

Color: 15 ppm, Pt-Co

Water: 0.03 wt. %

Solubility in Water: Nil

Appearance: Clear liquid

[1]Information derived from a letter from J. A. Larrabee dated August 22, 1979, Ref. No. 79AN800.

Table II
Composition* of EXXON DCPD-97, Lot CSTD BR #064-90-13 by Gas Chromatography[a]

Retention time, min.	Compound[b]	Area %[3]
1.02	2 methyl-1,3-butadiene[1]	0.94
1.22	cyclopentadiene[2]	5.60
8.76	tricyclo[3.2.1.0]dec-8-en[4]	0.11
9.74	endo dicyclopentadiene[5]	92.00
10.60	4-methyltricyclo[5.2.1.$0^{2,6}$]deca-3,8-diene[6]	0.57
10.90	10-methyltricyclo[5.2.1.$0^{2,6}$]deca-3,8-diene[7]	0.61
18.53	tricyclopentadiene (isomer 1)[8]	0.01
19.22	tricyclopentadiene (isomer 2)[9]	0.16

[a]Also 0.03 wt. % H_2O. For GC conditions see AID.25BA.79, Section 2.

[b]See Figure 11, AID.25BA.79, Section 2, for structures.

*Information from letter from J. A. Larrabee, dated August 22, 1979, Ref. No. 79AN800.

Table III
Chemical and Physical Properties of 1,3-cyclopentadiene[1]

Chemical Formula:	C_5H_6
CAS Registry Number:	542-92-7
Molecular Weight:	66.1
Melting Point:	-97.2°C
Boiling Point:	40.0°C
Density:	0.8021
@ 25°C and 760 mm Hg:	1 mg/liter = 370 ppm 1 ppm = 0.00270 mg/liter

[1]Information assembled by Toxicology Research Laboratory, Health and Environmental Research, DOW Chemical, November 18, 1975, "Literature Review and Published References Pertaining to the Toxicological Effects of Dicyclopentadiene."

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Table IV
Perkin-Elmer 3920B Gas Chromatograph: Conditions of Operation
Cyclopentadiene Nine-Day Vapor Inhalation Study

Column	1/4" x 5' stainless steel packed with 20% SP2100 on supelcoport 80/100 mesh
Temperatures	
Column	125°C
Injection port	150°C
Detector	150°C
Sample loop	Room temperature
Carrier, flow rate	Nitrogen, 40 mL/min
Hydrogen, pressure and flow rate	27 psi, 62 mL/min
Air, pressure and flow rate	50 psi, 550 mL/min
Sample size	1 mL vapor
Retention time	130 sec
GC attenuation	10 x 4
Detector	Flame ionization
Detection limit	0.02 ppm
Solvent for standards	n-hexane

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Table V
Parameters Examined During the Modified Irwin Screen
Cyclopentadiene Nine-Day Vapor Inhalation Study on Mice

Corneal Response	Tremors
Pupil Response	Convulsions
Tail Pinch	Salivation
Toe Pinch	Piloerection
Righting Reflex	Diarrhea
Locomotor Activity	Tail Elevation
Impaired Gait	Lacrimation
Respiration	Stereotypy

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Table VI
Time of Mortality - Cyclopentadiene LC50 Inhalation Study on Mice

Exposure Concentration	Number Died During or Immediately Following Exposure	Number Died on Days Post-Exposure 1	2	3 - 14	Total Dead/ Group Size
Male mice					
5465 ppm	3	3	-	-	6/6
2762 ppm	-	3	2	-	5/6
1427 ppm	-	-	2	-	2/6
Female mice (number dead)					
5465 ppm	-	6	-	-	6/6
2762 ppm	-	-	-	-	0/6
1427 ppm	-	-	-	-	0/6

Six-Hour LC_{50} Values

B6C3F1 Mice

Sex	LC50 (95% confidence limits)
Male	1778 ppm (1064-2972)
Female	3908 ppm (3021-5055)

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Table VII
Cyclopentadiene 9-Day Vapor Inhalation Study
Daily Mean Chamber and Animal Holding Room
Temperature and Relative Humidity

Exposure Day	Calendar Day	Mean Chamber Concentration								Animal Holding Room	
		2558 ppm		714 ppm		244 ppm		0 ppm (Control)		Room 146	
		Temp.[1]	% R.H.[2]	Temp.	% R.H.	Temp.	% R.H.	Temp.	% R.H.	Temp.	% R.H.
1	1	21.8	46.3	21.9	46.3	22.1	46.1	22.1	49.5	22.7	49.0
2	2	22.7	48.8	23.8	48.7	22.8	49.0	23.0	52.0	23.1	56.7
3	3	22.0	48.8	24.8	48.3	24.1	46.0	23.0	51.5	23.1	57.3
4	4	23.2	*	23.4	47.3	24.4	49.0	22.9	50.3	22.7	57.3
5	5	23.3	46.7	23.5	48.0	24.0	49.8	23.7	51.7	22.7	58.7
	6									22.7	54.0
	7									21.7	54.0
6	8	**	**	22.0	46.0	22.5	46.8	22.7	45.7	22.7	55.3
7	9	**	**	22.1	45.8	23.3	44.8	21.9	47.0	22.7	56.0
8	10	**	**	22.7	46.3	24.0	48.0	23.0	47.7	22.6	54.0
9	11	**	**	22.5	44.0	22.9	44.5	22.4	44.5	22.6	51.3
Means		22.6	47.5	23.0	46.7	23.3	47.1	22.7	48.9	22.7	54.9
SD[3]		0.7	1.2	1.0	1.5	0.8	1.9	0.5	2.8	0.4	2.8

[1]Temp. = Temperature (°C), daily mean value.

[2]% R.H. = Percent Relative Humidity, daily mean value.

[3]SD = Standard Deviation

*Could not see gauge.

**No value because all animals are dead.

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Table VIII
Daily Cyclopentadiene Concentrations in Exposure Chambers
Nine-Day Cyclopentadiene Vapor Inhalation Study

Exposure Day	Target Concentrations, ppm							
	2500		750		250		0	
	Measured Concentrations, ppm							
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
1	2580	148	763	93	245	23	2.39	1.25
2	2456	192	774	32	245	12	1.20	0.35
3	2532	27	808	36	255	13	0.50	0.19
4	2665	23	651	161	217	72	0.41	0.07
5	699[A]	870	812	55	230	70	0.23	0.10
6	*		682	54	232	22	0.39	0.52
7			655	102	194[B]	123	0.53	0.64
8			549	198	263	23	0.24	0.21
9			728	51	246	12	0.66	0.40
10			720	50	272	8	0.13	0.08
11			*		284	5	0.06	0.02
Mean	2187	(2558)[C]	714		244		0.61[D]	
SD	835	(88)	82		25		0.69	
CV	38	(3)	11		10			

A = Malfunction of the generator occurred approximately 1 hour after exposure started resulting in low concentrations (Total exposure time was 6 hours).

B = Since the generator temperature was found to be too high, the delivery of the test material was stopped for about 1 hour. All measured concentrations were included in statistics.

C = The numbers in the parentheses do not include the 699 ppm value in the statistics.

D = For the first 2 days, the control air intake was too close to the CPD vapor generator. [Note: It was found in a later study that the control values would be closer to the "0" level if there were no carry-over from the previous sample. Detection limit is approximately 0.02 ppm.]

SD = Standard deviation

CV = Coefficient of variation in %

* = Exposure terminated because all animals were dead.

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Table IX
Daily Dicyclopentadiene Concentrations in Exposure Chambers
Nine-Day Cyclopentadiene Vapor Inhalation Study

Exposure Day	Mean Chamber Concentration of Cyclopentadiene, ppm		
	2558	714	244
	Concentration of Dicyclopentadiene, ppm		
1	< 2 (two samples)*	Not measured	Not measured
2	< 2 (approx. 6 hrs)	< 0.5	< 0.5
3	< 2 (approx. 6 hrs)	< 0.5	< 0.5
4	< 20 (approx. 1.5 hr)**	< 0.5	< 0.5
5	> 200 (approx. 5 hrs)[A]	< 0.5	< 20 (approx. 3 hrs)**
6	No exposure	< 0.5	< 20 (approx. 1 hr)**
7	No exposure	< 0.5	-[B] (approx. 0.5 hrs)**
8	No exposure	< 20 (approx. 0.5 hrs)**	< 0.5
9	No exposure	< 0.5	< 0.5
10	No exposure	< 0.5	< 0.5
11	No exposure	No exposure	< 0.5

*After 2nd exposure day, approximately 7 to 10 DCPD analyses were made daily.
**Rest of the exposure, the concentration was < 0.5 ppm.
A = Liquid DCPD was found in generator which caused the high concentration. Exposure on this day was to only one surviving mouse.
B = Generator temperature was too high, and two unknown peaks were observed.

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Table X
Time of Mortality
Nine-Day Cyclopentadiene Vapor Inhalation Study on Mice

Exposure Concentration	Completed Number of Exposures 1	2	3	4	5	6 to 9	Total Dead/ Group Size
			Male mice (number dead)				
2558 ppm	10	-	-	-	-	-	10/10
714 ppm	-	4	6	-	-	-	10/10
244 ppm	-	-	-	-	-	-	0/10
0 ppm	-	-	-	-	-	-	0/10
			Female mice (number dead)				
2558 ppm	-	-	2	7	1	-	10/10
714 ppm	-	-	2	3	2	3	10/10
244 ppm	-	-	-	-	-	-	0/10
0 ppm	-	-	-	-	-	1*	1/10

*Accidental cage death.

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Table XI
Body Weight Changes for Male B6C3F1 Mice During
the Nine-Day Cyclopentadiene Vapor Inhalation Study

	Mean Chamber Concentration			
	2558 ppm	714 ppm	244 ppm	0 ppm
Completed Exposure	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	Body Weight, g			
0	20.07 ± 1.17	19.89 ± 0.94	19.85 ± 0.94	20.07 ± 1.22
	Body Weight Change from Day 0, g			
1	*	-0.60[a] ± 0.72	0.49 ± 0.42	0.93 ± 1.46
4	*	*	0.81 ± 0.89	0.57 ± 0.78
5	*	*	1.87 ± 0.91	1.64 ± 0.50
6	*	*	1.98 ± 0.81	2.21 ± 0.76
9	*	*	2.62 ± 1.32	2.79 ± 1.03

SD = Standard Deviation

a = $0.05 > P > 0.01$

*All mice were dead

N = 10 mice/group (at first exposure)

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Table XII

Body Weight Changes for Female B6C3F1 Mice During the Nine-Day Cyclopentadiene Vapor Inhalation Study

	Mean Chamber Concentration			
	2558 ppm	714 ppm	244 ppm	0 ppm
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Completed Exposures	Body Weight, g			
0	17.76 ± 0.85	17.04 ± 1.37	17.31 ± 0.91	17.12 ± 1.58
	Body Weight Change from Day 0, g			
1	-0.23 ± 0.57	0.06 ± 0.55	0.55 ± 0.58	-0.01 ± 0.71
4	*	-0.25[a] ± 0.71**	1.17 ± 0.86	0.76 ± 0.75
5	*	0.80 ± 0.87**	1.74 ± 0.77	1.84 ± 0.99
6	*	0.77 ± 0.83**	1.77 ± 0.68	1.66 ± 0.80
9	*	*	2.50[a] ± 0.69	1.56 ± 0.86

SD = Standard Deviation

a = 0.05 > P > 0.01

*All mice were dead

**Data based on only 3 or 4 animals because all others had died following the first exposure period.

N = 10 mice/group (at first exposure)

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Table XIII
Food Consumption for Male and Female B6C3F1 Mice
During the Nine-Day Cyclopentadiene Vapor Inhalation Study

	Mean Chamber Concentration			
	2558 ppm	714 ppm	244 ppm	0 ppm
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	Male B6C3F1 Mice			
4 days pre-exposure	5.21* ± 0.75	5.24 ± 0.50	5.23 ± 0.99	5.16 ± 0.85
4 completed exposures	**	**	3.94 ± 0.45	4.05 ± 0.38
9 completed exposures	**	**	3.32 ± 1.07	3.58 ± 1.60
	Female B6C3F1 Mice			
4 days pre-exposure	5.20 ± 0.29	4.89 ± 0.57	4.89 ± 0.75	4.12 ± 0.29
4 completed exposures	**	**	3.81 ± 0.60	3.77 ± 0.67
9 completed exposures	**	**	4.18 ± 0.62	3.65 ± 0.75

SD = Standard Deviation

N = 10 per group (at first exposure).

*Values represent gm/mouse/day.

**Significant number of mice died prior to food consumption measurement; consequently, value was not calculated.

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TABLE XIV

Organ Weights for Male and Female B6C3F1 Mice
Following the Cyclopentadiene Nine-Day Vapor Inhalation Study

Parameter	Mean Chamber Concentration			
	2558	714	244	0
			Mean ± SD	Mean ± SD
			Male Mice	
Mean Liver Weight, grams	*	*	1.4803 ± 0.1642	1.5101 ± 0.2253
Mean Liver Weight as % Body Weight	*	*	6.4740 ± 0.5288	6.3551 ± 0.5957
Mean Kidney Weight, grams	*	*	0.4163 ± 0.0260	0.4364 ± 0.0470
Mean Kidney Weight as % Body Weight	*	*	1.8235 ± 0.0832	1.8445 ± 0.1576
Mean Lung Weight, grams	*	*	0.1363 ± 0.0168	0.1478 ± 0.0300
Mean Lung Weight as % Body Weight	*	*	0.5979 ± 0.0748	0.6244 ± 0.1210
Mean Testes Weight, grams	*	*	0.1829 ± 0.0140	0.1834 ± 0.0235
Mean Testes Weight as % Body Weight	*	*	0.8020 ± 0.0634	0.7760 ± 0.0930
			Female Mice	
Mean Liver Weight, grams	*	*	1.3090[b] ± 0.1241	1.1489 ± 0.0966
Mean Liver Weight as % Body Weight	*	*	6.5887[b] ± 0.4880	5.9136 ± 0.4992
Mean Kidney Weight, grams	*	*	0.3023 ± 0.0159	0.2997 ± 0.0304
Mean Kidney Weight as % Body Weight	*	*	1.5243 ± 0.0899	1.5402 ± 0.1298
Mean Lung Weight, grams	*	*	0.1378 ± 0.0105	0.1679 ± 0.0839**
Mean Lung Weight as % Body Weight	*	*	0.6947 ± 0.0524	0.8641 ± 0.4372**

SD = Standard Deviation

1 mice died prior to sacrifice.

**Includes high value (0.386 g) for one animal. Without this value, mean ± SD is 0.1406 ± 0.0201 for absolute weight and 0.7209 ± 0.0859 for % body weight. No statistical difference when value was excluded.

b = $0.01 > P > 0.001$

N = 10 per group (at first exposure).

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Appendix A

CURRENT STATUS OF KNOWLEDGE ABOUT THE TOXICITY OF CYCLOPENTADIENE

H. F. Smyth, Jr.
April 8, 1980

Information on the toxicity of Cyclopentadiene was sought in the laboratory's previous studies, its data files, and by a March 4, 1980 search on Toxline, Toxback, Mekline and Cancerline. Little was found.

In 1941, this laboratory (1) studied repeated inhalation by rats of vapors evolved from a dicyclopentadiene adduct, presumed on good evidence to be cyclopentadiene. Concentrations were measured by activiated charcoal adsorption. Thirty 4-hour inhalations of 400 ppm had minor effects: growth slightly retarded, light cloudy swelling of liver and kidney, and light lung congestion.

Unpublished work is cited as justification for the TLV of 75 ppm (2), stated to guard primarily against upper respiratory tract irritation. Thirty-five 7-hour exposures of rats to 500 ppm caused only mild cloudy swelling in the liver and kidney tubular epithelium. One hundred thirty-five 7-hour exposures of rabbits, rats, guinea pigs and dogs to 250 ppm caused no detected effects. Six-hour exposures of dogs, 29 times at 400 ppm, the 15 at 800 ppm had no ill effects. Human sensory response was reported to be "distinctly unfavorable" at 250 ppm. This latter datum is all that appears in the 1978 NIOSH Registry (3), being entered as "human LCLo".

Shashkina (4) reported the LC50 to be 0.59 millimols. It is in accord with repeated inhalation results cited above if this is millimols per leter, 39 mg/l, equivalent to 14,400 ppm, although Russian work usually expresses concentrations in units per cubic meter. Added to this ambiguity, the abstract does not mention species or time of inhalation. The LC50 datum does not seem sufficiently important to justify obtaining and translating the Russian original. Long term exposures are mentioned in the abstract, but no concentrations are given. Effects noted were changes in peripheral blood composition, a rise in the threshold of neuromuscular excitability and inflammatory and sclerotic changes in the lungs. A maximum permissible concentration of 5 mg/m^3 is suggested.

In summary, cyclopentadiene appears to be a substance with only moderate chronic inhalation toxicity for experimental animals. Effects are chiefly irritation in liver, kidney and lung. Upper respiratory tract irritation to humans appears to be more objectionable than is internal toxicity.

Appendix A
Bibliography

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2. Documentation of Threshold Limit Values for Substances in Workroom Air. Amer. Conference of Governmental Industrial Hygienists, Cincinnati, Ohio. 1971, p. 66-67.

3. 1978 NIOSH Registry of Toxic Effects of Chemical Substances. U.S. Dept. of Health, Education and Welfare. U.S. Gov't. Printing Office, June 1979.

4. Shashkina, L. F. The maximum permissible concentration of cyclopentadiene and dicyclopentadiene in the atmosphere of industrial premises. Gigena Trude i Proh. Zahdevaniya., 9(12), 13-19, 1965. From Chem. Abstracts, 64, 20509c.

UNION CARBIDE

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Quality Assurance Unit Study Inspection Summary

Test Substance: Cyclopentadiene

Study: Six-Hour LC50 and Nine-Day Vapor Inhalation Study on Mice

Study Director: [redacted]

The Quality Assurance Unit of BRRC conducted the inspections listed below and reported the results to the study director and to management on the dates indicated. It is the practice of this Quality Assurance Unit to report the results of each inspection to both the study director and management.

Inspection Date	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
5-31-79	Protocol	5-31-79	5-31-79
10-12 to 10-18-79	Final Data	10-18-79	10-29-79
9-1-81	Event - Final Report	9-1-81	10-9-81
1[illegible]-4 to 11-6-81	Final Report	11-6-81	11-16-81

[redacted]
Quality Assurance Officer

11/16/81
Date

LJC:acc

[redacted]

[redacted]

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AMGR.0094.77

BIODEGRADATION OF m-PHENOXYBENZOIC ACID, PENTAERYTHRITOL AND METHANESULFONYL CHLORIDE IN THE PRESENCE OF A SOFT CO-SUBSTRATE

by

[redacted]

IN 79124 Code 50070799

[redacted]

Koninklijke/Shell-Laboratorium, Amsterdam
(Shell Research B.V.)

AMGR.0094.77

BIODEGRADATION OF m-PHENOXYBENZOIC ACID, PENTAERYTHRITOL AND METHANESULFONYL CHLORIDE IN THE PRESENCE OF A SOFT CO-SUBSTRATE

by

[redacted] and C.J.M. WOLFF

IN 79124

Code 50070799

[redacted]

CONFIDENTIAL

Throughout this report the words "Shell" and "Group" are used collectively in relation to companies associated together under the name of the Royal Dutch/Shell Group of Companies.

AMGR.0094.77

BIODEGRADATION OF m-PHENOXYBENZOIC ACID, PENTAERYTHRITOL AND METHANESULFONYL CHLORIDE IN THE PRESENCE OF A SOFT CO-SUBSTRATE

by

[redacted]

IN 79124

Code 50070799

Approved by: H.B. van der Heijde

SUMMARY

Organic chemicals which seem non-biodegradable in the standard BOD test are sometimes readily broken down upon the addition of a soft co-substrate. This effect has been encountered with m-phenoxybenzoic acid, pentaerythritol and methanesulfonyl chloride.

March 1977

CONTENTS

BIODEGRADATION OF m-PHENOXYBENZOIC ACID, PENTAERYTHRITOL AND METHANESULFONYL CHLORIDE IN THE PRESENCE OF A SOFT CO-SUBSTRATE

1. INTRODUCTION

Organic chemicals which may show up in aqueous effluents from new manufacturing processes are nowadays screened with respect to their biodegradability. At KSLA this is done routinely in respirometric Biochemical Oxygen Demand (BOD) tests. When a compound is not broken down under those conditions it is subsequently subjected to what is called a BOD inhibition test. The purpose of this test is to establish whether the compound is simply non-biodegradable or if it is also toxic to bacteria in the sense that it inhibits the microbial degradation of a soft substrate.

During inhibition testing it is sometimes observed that more oxygen is consumed than may be attributed solely to the soft substrate. In such a case it must be concluded that the non-biodegradability of the compound in the initial BOD test has only been apparent and that degradation may occur if stimulated by additional substrates and/or nutrients.

This phenomenon in itself is well known. We were surprised, however, to find that the following three compounds were degraded during inhibition testing:

m-phenoxybenzoic acid	$C_6H_5OC_6H_4COOH$
pentaerythritol	$C(CH_2OH)_4$
methanesulfonyl chloride	CH_3SO_2Cl.

The fact that these compounds are important base materials/intermediates in recently developed Shell processes prompted us to report this experience separately.

2. METHODS AND PROCEDURES

2.1. BOD measurement

The BOD is measured in a respirometer at 20 °C on a $\frac{1}{2}$ l volume of a solution of a compound in a mineral medium. The compound is added to a Theoretical Oxygen Demand (ThOD, computed for complete mineralization) of 40 - 60 mg/l. The mixture is inoculated with 10 %v of filtered river water. The mineral medium is a solution in demineralized water of: $FeCl_3.6H_2O$ 0.38 mg/l, $MgSO_4.7H_2O$ 34 mg/l, NH_4NO_3 50 mg/l, KH_2PO_4 85 mg/l, K_2HPO_4 217 mg/l and Na_2HPO_4 266 mg/l. Nitrification is prevented by the addition of 0.50 mg/l of allylthiourea. The pH of the medium is 7.6 with this composition.

2.2. BOD inhibition test

The inhibition is measured in the same BOD respirometer on solutions of 50 mg/l glucose and 4 mg/l bacto-peptone in filtered river water which has been enriched with 1 mg/l P, as Na_2HPO_4, and 10 mg/l N, as $NaNO_3$. The compound tested is added to this solution in a series of concentrations: 10, 100 and 1000 mg/l.

3. RESULTS

3.1. m-Phenoxybenzoic acid

In the initial BOD test no oxygen was taken up within 15 d: see curve 1 in Fig. 1. In the blank inhibition test glucose and peptone were readily degraded with an oxygen uptake of, as usual, from 66 % after 5 d to 86 % after 15 d of their combined ThOD. See curve 2 in Fig. 1.

In subsequent inhibition tests with 10 and 1000 mg/l m-phenoxybenzoic acid added, the oxygen consumed was no more than that attributable to the degradation of glucose and peptone. But at 100 mg/l the O_2 uptake resumed after 15 d and went on till the end of the bacterial growth phase, i.e. a further 4 d. The oxygen taken up during the latter period amounted to 60 % of the ThOD of 100 mg/l m-phenoxybenzoic acid, and it may therefore be assumed that at that time the compound had been metabolized completely. The rate of oxygen uptake in the 100 mg/l experiment is represented by curve 3 in Fig. 1.

In a second BOD test on m-phenoxybenzoic acid, in the absence of glucose-peptone, 50 ml inoculate was taken from the above successful inhibition test. As is shown by curve 4 in Fig. 1 degradation of the compound started after a lag phase of 3 d and the growth phase ended after another 4 d. The test was continued for 15 d and at that time 85 % of the ThOD of the compound had been taken up.

To check whether the above adaptation can be induced at will, the whole procedure was repeated. In the inhibition test with glucose and peptone and with fresh river water as inoculum, 100 mg/l m-phenoxybenzoic acid was again completely degraded. This time, however, in the BOD test with seed from the second inhibition test, oxygen was not taken up to any measurable extent in the absence of glucose and peptone.

It seems worth while to mention here that m-phenoxybenzaldehyde was also completely degraded in a BOD test inoculated with the culture which had adapted to m-phenoxybenzoic acid in the inhibition test on the latter compound. m-Phenoxybenzaldehyde had not been broken down in BOD and BOD inhibition tests on the compound proper.

3.2. Pentaerythritol

Our experience with this compound was very similar to that with m-phenoxybenzoic acid. In the inhibition tests pentaerythritol was degraded at all three concentrations, 10, 100 and 1000 mg/l, starting after 12 - 16 d. This time the compound was not degraded in the first BOD test (without the co-substrate) with adapted seed but was in a second test. The degradation curves are presented in Fig. 2.

3.3. Methanesulfonyl chloride

With this compound the results obtained were much the same. In an initial BOD test no oxygen was taken up. Subsequently, in inhibition tests with 10 and 100 mg/l of methanesulfonyl chloride plus glucose and peptone, degradation was complete. In the further BOD test with adapted seed the compound was degraded to 64 % of its ThOD after a lag time of 11 d. The respective curves are shown in Fig. 3.

4. DISCUSSION

The tests described have shown that m-phenoxybenzoic acid (with an ether -C-O-C- bond), pentaerythritol (with a quaternary C atom) and methanesulfonyl chloride (a one-carbon compound) are biodegradable, at least after adaptation of the micro-organisms. The work also showed that simply the addition of a soft co-substrate may be sufficient to induce adaptation.

We do not claim that a glucose/peptone/river water medium is optimal for the inducement of adaptations. However, the fact remains that with this medium adaptation of a mixed culture to three rather remarkable compounds has occurred.

Amsterdam, March 1977
JYW/BJ

FIGURES 1,2 AND 3

AMGR 0094.77

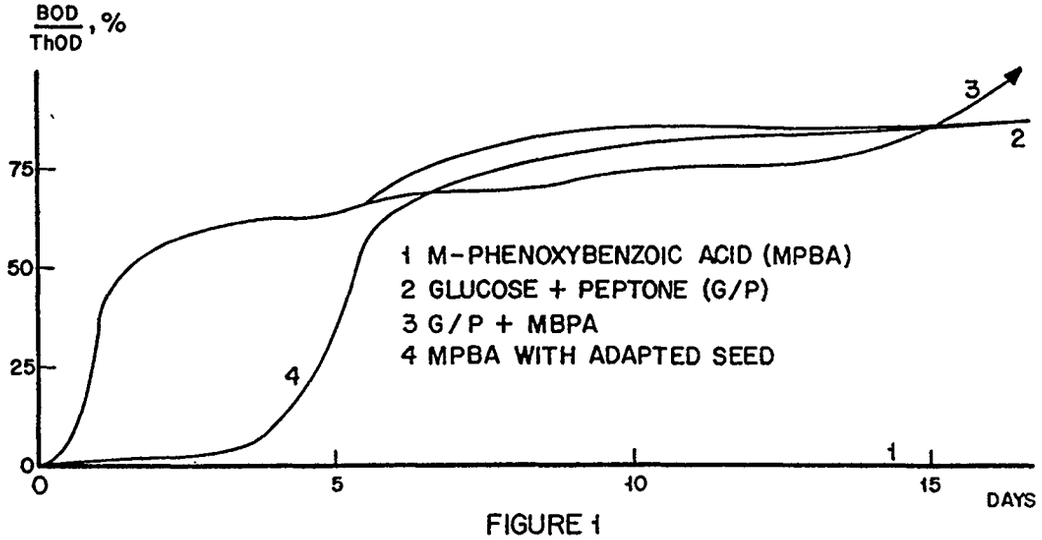

FIGURE 1

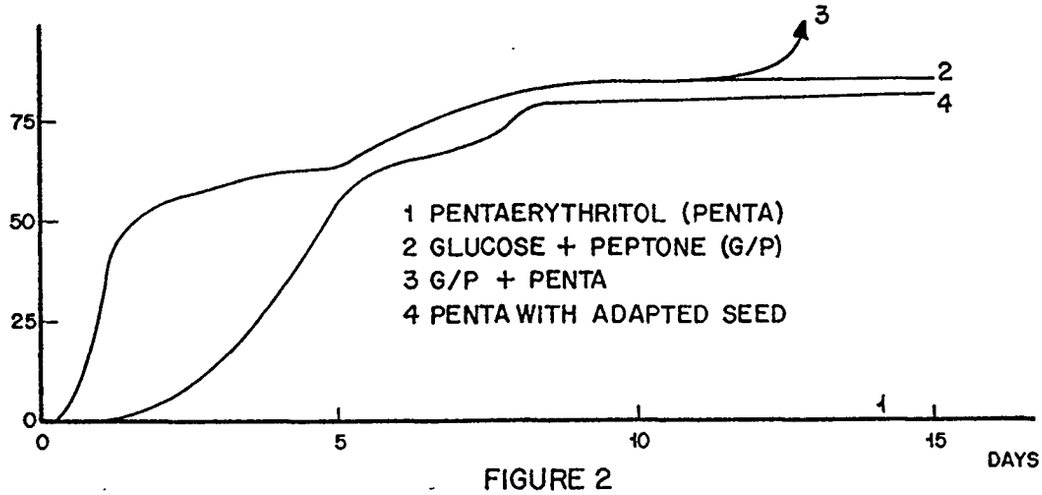

FIGURE 2

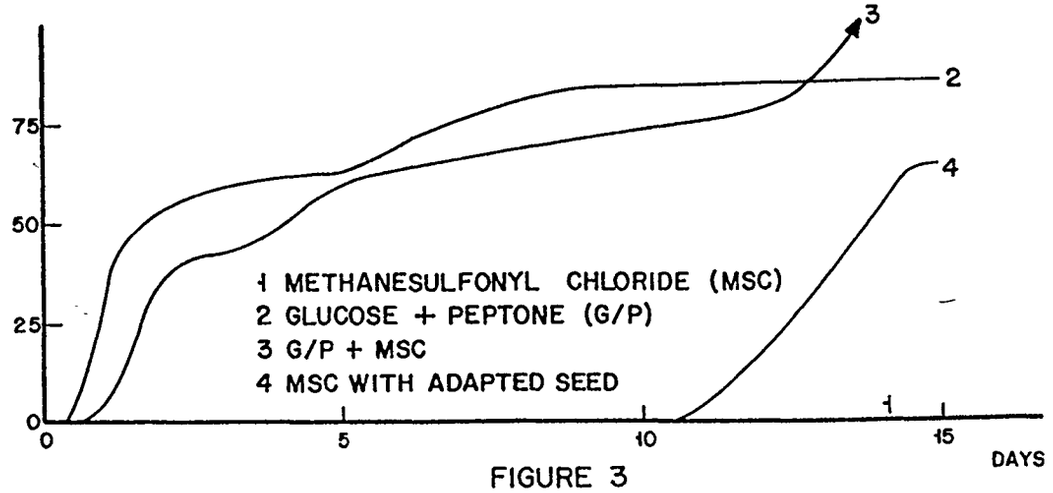

FIGURE 3

DRAWN GL
DWG 77-07-108

BOD AND BOD INHIBITION CURVES

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